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BASE-PROTEIN-ACID COMPOUNDS PREPARED FROM FIBRIN

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I

These pages extend remarks made earlier[1] wherein biologic and chemical evidence was cited which proves protoplasm (including blood, lymph, milk and egg white) not a mere mixture of protein with salt and water. The physical and chemical characteristics of living matter are repeated only when a chemical unit is made of the three. Thereafter it was shown, in the instance of casein, how such hydratable base-protein-acid compounds might be prepared. What is requisite is that the necessary chemical reactions be permitted to proceed as under the conditions existent in protoplasm, wherein the average water content of 80 per cent is all held in combined form. In other words, no "free" water may be present in the reaction mixture. The following paragraphs show how such triple compounds may be prepared from the fibrin of blood.

"Pure" fibrin absorbs little water (its capacity to "swell" is low). Enormous increase occurs if either an alkali or an acid is added, because the resultant proteinates have great hydration values.[2] Neither of these compounds alone, however, may be taken as the chemical analogue of fibrin as it exists in nature, where both acid and alkali appear in it (as the commonly designated mixture of "salts" ashable out of it), in the approximate relation of 0.7 of the former to 1 of the latter.

The "pure" fibrin employed in the experiments described here was obtained by first bringing a commercial fibrin into "solution" (really into a state of hydration miscible with more water) in strong potassium hydroxide. After this mixture had been filtered, the fibrin was reprecipitated through neutralization with strong hydrochloric acid. After

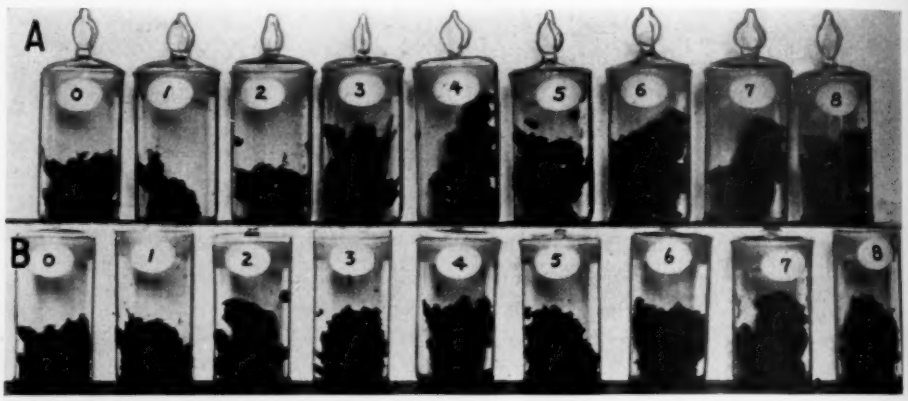
From the Laboratory of Physiology, University of Cincinnati.

1. Fischer, M. H., and Suer, W. J.: Arch. Path. **20**:683, 1935.

2. Fischer, M. H.: Oedema and Nephritis: A Critical, Experimental and Clinical Study of the Physiology and Pathology of Water Absorption by the Living Organism, ed. 3, New York, John Wiley & Sons, Inc., 1921, p. 61.

several washings with water it was covered with 95 per cent alcohol and stored under it.

Example: One hundred grams of powdered fibrin was allowed to swell for twenty-four hours in the ice box in 1,000 cc. of water, after which 67.5 Gm. of pure potassium hydroxide dissolved in 500 cc. of water was added. The half-gelatinous mixture was permitted to stand twenty-four hours, and then, to render the mixture more liquid, 500 cc. of water was added and the whole filtered. Concentrated hydrochloric acid was then slowly stirred into the filtrate until maximal precipitation was obtained. When 81.1 cc. had been added, the supernatant liquid was faintly acid to litmus. It was decanted, and the precipitated fibrin was washed throughout a day with several changes of water, after which the moist precipitate was covered with three volumes of 95 per cent alcohol. After standing another day, the precipitate was filtered off, washed with more alcohol and preserved under it. Such fibrin was still highly solvated. From the alcohol-wet yield of 126 Gm. in the example cited, an aliquot portion showed 33.14 Gm. to be



A shows how hydrochloric acid in increasing quanta may be added to the 75 per cent hydrated potassium fibrinate jelly shown in 0 without visible effect on its physical characteristics. *B* illustrates the same observation when a definite quantity of each of a series of different acids was added to the standard potassium fibrinate gel shown in 0.

solid (26.3 per cent). Nor is it to be considered absolutely pure, for on ashing we still recovered 0.365 per cent potassium chloride (which, however, was not merely adherent to but an intimate part of the protein).

To prepare potassium fibrinate, we separated the 126 Gm. of solvated fibrin, obtained as described, from its surrounding fluid by filtration. In the process the wet weight shrank to 102 Gm. To the resultant, 25 cc. of water and 13 cc. of twice normal potassium hydroxide were added. After twenty-four hours the clear solid jelly shown in tube 0 of *A* in the figure was obtained. Counting in any alcohol remaining, one may say that this product did not carry more than 75 per cent of water (not more, therefore, than the physiologic maximum of tissue).

The remaining tubes of *A* show how *strong hydrochloric acid may be added to this potassium fibrinate gel without change in its physical*

character. The acid does not strike the base off the proteinate but adds itself to the protein when, as here, free water is absent. Not until tube 8 is reached, in which the amount of acid added exceeds 70 per cent of the base present in the proteinate, does combination with the base occur and precipitation of fibrin in a "neutral" and less hydrated form come about (the gel turns white).

B illustrates the production of such "triple" compounds from several different acids. The control potassium fibrinate gel with 75 per cent water is again shown under 0. In the remaining tubes, tartaric, hydrobromic, citric, acetic, lactic, hydrochloric, phosphoric and sulfuric acids have been added in this order in chemically equivalent amounts up to 69 per cent of the alkali contained in the original compound. The colloid character of the gel was in no wise altered except in the last two tubes (to understand this it needs to be recalled that protein phosphate and protein sulfate stand lowest in *absolute* hydration value in any protein series so far studied).

II

To be brief, we condense some further observations on the fibrin compounds described here (and the caseinates noted earlier) into the following paragraphs.

The alkali proteinates or the acid proteinates of either take up more water ("swell more") than the proteins alone. *Their hydration capacities* are thus increased. But specific differences appear as different bases or different acids are employed. The absolute hydration values are greatest with potassium, ammonium and sodium, much lower with the earth metals and lowest with the heavy metals. In an acid series, the compounds with hydrochloric, hydrobromic and lactic acid stand at the top, the compounds with various organic acids lie lower and the compounds with phosphoric and sulfuric acids bring up the bottom. Thus the so-called "physiologic" degree of hydration characteristic of living matter (its normal water content) may be assigned to the mixture and the relative proportions of the various bases and acids that appear in it (the relative quantities and qualities of its "salts"); and the hydrating or dehydrating effects of pharmacologically applied bases, acids or salts, to the additive or substitution effects which the constituent radicals have on the substrate's first composition. So while all alkalis and acids increase the swelling of a protein not previously saturated (this is the case, too, for all "normal" protoplasm), all salts (with the exception of ammonium and possibly potassium) tend uniformly to bring about dehydration. But at the same "osmotic" concentration, their relative effectiveness is very different, the sequence of the different alkali or acid radicals being that given.

The different alkali proteinates show, too, *a different miscibility with water* (as ordinarily put, a different "solubility" in water). Only the

light metal proteinates mix easily with more. And this they do as does egg white or blood plasma or tissue juice, in all of which (as "neutral" to indicators as the alkali proteinates described here when their hydration capacity has not been exceeded) pronounced "alkalinity" develops (due to hydrolysis) when more water is added. This miscibility continues if a "physiologic salt solution" (eighth to sixth molar sodium chloride) is employed instead of water, but the development of end "alkalinity" is then much reduced. The acid proteinates behave like the alkali compounds; only, when they are mixed with water, the end reaction is acid instead of alkaline.[3]

What is the behavior of base-protein-acid compounds under these descriptive heads? *The introduction of increasing increments of acid into an alkali proteinate reduces progressively both its capacity to swell and its miscibility with water or salt solution.* A protein saturated with both base and acid (as the potassium-fibrin-chloride shown in tube 7 of *A*) fails to mix with water or a salt solution as completely as a beefsteak. But the base-protein-acid compounds also all take up a defined quantum of water. If not completely "saturated" with either alkali or acid, they "swell" when more of either is added (as do living cells when made edematous by such treatment). In neutral salt solutions the triple compounds shrink (are dehydrated) as the concentration of any nonreactive salt about them is increased. Herein their behavior parallels the so-called "osmotic" behavior of individual cells and tissues toward similar "hypertonic" solutions. But if the added salt is of the heavy metal variety or is possessed of a greatly dehydrating acid radical, all osmotic rules are off and the low hydration capacity of the ultimately formed compound dominates the picture. (These are the effects of those "salts" which have always proved "exceptional" in their osmotic behavior when tested on living cells.)

Regarded collectively, therefore, the properties of these base-protein-acid compounds are such that they register large resistance (meaning no visible change in physical properties) to rather large change in surroundings. They are "buffered" against the action of water, of various salts and of acids and alkalis even as are living cells. In biologic terms, their "factor of safety" is large against "injury."

SUMMARY

Base-protein-acid compounds have been produced from fibrin. Their "behavior" toward water, salts, acids and alkalis is described. It parallels qualitatively and quantitatively the reaction of living cells to like changes in surroundings.

3. How this explains the origin of acid or alkaline secretions from neutral secretory glands (salivary, pancreatic, gastric, sudorific or renal) has been touched on before (Fischer, M. H.: Soaps and Proteins: Their Colloid Chemistry in Theory and Practice, New York, John Wiley & Sons, Inc., 1921, p. 78).

FIBRIN AS CATALASE

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I

It has been shown in previously published articles that various proteins extractable from protoplasm yield gelatinous systems, through treatment with alkalis, acids [1] or both together,[2] which display a varying hydratability, high electrical resistance, great viscosity and optical behavior identical with the similar properties of living matter. More recently a report on their solvent characteristics was added.[3] It was shown that protein hydrates "dissolve" various materials less soluble in equal volumes of water or insoluble in it. This added proof that protoplasm is free from ordinary water was then used to explain why so many dehydrolyses (syntheses) occur in it which in the alimentary tract (where "free" water is present) appear only as hydrolyses (analyses or "digestions").

A number of chemical set-ups that fortify the rule were described. The following is picked for illustration:

$$\text{ester (fat)} + H_2O + H_2SO_4 \rightleftarrows \text{fatty acid} + \text{alcohol} + H_2SO_4$$

This much studied reaction proceeds left or right (with synthesis or analysis *complete!*) depending solely on the absence or the presence of free water in the total system. Consideration of the equation reveals these facts: In the synthetic half the sulfuric acid acts not only (*a*) to bind any water liberated in the reaction (thus keeping the total system anhydrous) but (*b*) as its "catalyst."

Applied to living matter (or to the chemical fractions extractable from it), this generalization makes evident that any of its various "hydrophilic colloids" (all its proteins, all its carbohydrates and even some of its "peculiar" fats, therefore) act as does the sulfuric acid in the foregoing equation. Thus any or all of them may be considered

From the Laboratory of Physiology, University of Cincinnati.

1. Fischer, M. H.: Oedema and Nephritis: A Critical, Experimental and Clinical Study of the Physiology and Pathology of Water Absorption by the Living Organism, ed. 3, New York, John Wiley & Sons, 1921, p. 151. Fischer, M. H., and Hooker, M. O.: The Lyophilic Colloids, Springfield, Ill., Charles C. Thomas, Publisher, 1933, p. 230.

2. Fischer, M. H., and Suer, W. J.: Arch. Path. **20**:683, 1935.

3. Fischer, M. H., and Suer, W. J.: Arch. Path. **26**:51, 1938.

active in the establishment of the proper milieu for the activity of any ferment of the "dehydrolase" [4] type. These paragraphs emphasize that in addition *they may be the "enzyme."* Thus we try to make "catalysis" as the name of an *activity* into the name of a *thing;* we attempt to redefine "protoplasm," heretofore regarded merely as the *seat* of ferment activity, as the *ferment itself.* Universality of distribution and overweight in tissue analysis fix a primary interest on the *proteins* of living matter.

II

The capacity of lymph, blood or tissue cells to decompose hydrogen peroxide is historic. Thenard in 1818 described the effects of metals, oxides and "fibrin" on the reaction. Besides its practical employment for decades past (as in the treatment of wounds), the reaction has come to underlie every theory of "catalysis" (Berzelius, 1835) and of "fermentation" (Schönbein, 1863) proposed since.[5]

The reaction $H_2O_2 = H_2O + O$ is accelerated by an almost universally present extractive of tissue to which the name "catalase" has been assigned. It is active in minimal concentration, is recoverable from the end products of the reaction, brings about an "unlimited" amount of decomposition and is destroyed by heat, various "poisons" and other agents, wherefore it has long been assigned to that group of catalysts of protoplasmic origin accepted today as the "ferments." The next paragraphs tend to show that a simple protein (specifically the fibrin of blood) under biologic circumstances behaves in every way like catalase.

III

The fibrin clot whipped out of spontaneously coagulating bovine blood, well washed in water and dried, and presenting thus the ordinary "commercial" product of the chemists' shelves, does not, when added to a dilute hydrogen peroxide mixture, decompose it. As some might put the matter, it is without catalase activity and "dead." The material is not easily brought back into "solution" (really, into a state of hydration miscible with water). In most acids or alkalis it merely swells.[6] But when it is soaked in concentrated potassium hydroxide (less obviously in ammonium hydroxide) for a day or two in the ice box and stirred, a thick slime of uniform composition is obtained which can be diluted with more water and filtered through a coarse filter. The "dissolved" fibrin may be precipitated again by neutralization with hydrochloric acid. By this method we [7] "purified" (with great loss,

4. Carl Oppenheimer's term.

5. The story is best recited in Wilhelm Ostwald's Nobel-laureated thirty-two page monograph entitled "Ueber Katalyse" (Leipzig, S. Hirzel, 1902).

6. Fischer, M. H., and Moore, G.: Am. J. Physiol. **20**:330, 1907.

7. Fischer, M. H., and Suer, W. J.: Arch. Path., this issue, p. 811.

because of "digestion"?) a commercial fibrin (already of the rather low ash content of 0.709 per cent) to serve as the stock for the experiments now to be described.

The history of one of the several batches utilized in the experiments to be described is as follows: Two hundred grams of dry fibrin was powdered and allowed to swell twenty-four hours in the ice box in 2,000 cc. of water, whereafter 135 Gm. of pure potassium hydroxide dissolved in a liter of water was added to the gelatinous mixture. After two days more of soaking in the ice box, the resultant thick soup was filtered. Now concentrated hydrochloric acid (107.5 cc.) was slowly added until maximal precipitation of the fibrin was obtained. The supernatant liquid, neutral to litmus, was decanted and the precipitate washed a second time with water. After the supernatant fluid was again decanted, the residue was dehydrated by the addition of 3 volumes of 95 per cent alcohol. This was filtered off, and the white precipitate was washed with more alcohol and preserved under it. Thus 196 Gm. of precipitate was obtained, which was found by analysis to be 41.5 per cent solid.

The total product was treated with dry potassium hydroxide (3.46 Gm.) and the whole diluted by the addition of 500 cc. of water to a manageable syrup. The mixture was neutral to phenolphthalein until more water was added; then it turned alkaline.

Such readily reproducible proteinates were the "standard" from which the experiments now to be described proceeded. Though essentially pure potassium fibrinate, they still carried some chlorine (also chemically bound to the proteins for reasons elucidated elsewhere [2]).

A quantitative study of the decomposition of hydrogen peroxide by such materials under identical circumstances was accomplished via a battery of gas-washing bottles of uniform design, connected by a delivery tube to a pneumatic trough holding calibrated receptacles for the reception of gas. By such a scheme did Burge [8] study quantitatively the catalase content of tissue. An interesting parallel between his studies and ours lies in the fact that the "concentrations" of tissue, of hydrogen peroxide and of other factors employed by him are of the same order of magnitude as those used by us.

IV

Pure (potassium) fibrinate is without catalytic activity. Alone or diluted with water, it fails to decompose any of the 5 cc. of 30 per cent hydrogen peroxide added to it (tube 1 of table 1). "Activation" is accomplished by the addition of alkali, as tubes 2, 3 and 4 illustrate.

It is not the alkali added which decomposes the hydrogen peroxide. This is demonstrated in table 2. Fibrinate, therefore, is "catalase," as an equivalent of alkali added to water is not.

8. Burge, W. E.: Am. J. Physiol. **41**:153, 1916.

For maximal yield of oxygen an optimal concentration of alkali is necessary. As ordinarily put, correct p_H is called for. This is shown in table 1. The fact is reillustrated in table 3. Here sodium hydroxide has replaced potassium hydroxide, and a higher concentration of sodium hydroxide is needed to give a maximal amount of gas. Though the

TABLE 1.—*Experiment Demonstrating That Pure (Potassium) Fibrinate Is Without Catalytic Activity Until Alkali Is Added to It*

Tube	Mixture*	Cubic Centimeters of Gas Yielded in Given Number of Minutes			
		15 Min.	30 Min.	45 Min.	60 Min.
1	5 cc. fibrinate + 40 cc. H_2O (control)	0	0	0	0
2	5 cc. fibrinate + 39.5 cc. H_2O + 0.5 cc. 2N KOH	85	130	150	165
3	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH	100	150	180	200
4	5 cc. fibrinate + 38 cc. H_2O + 2 cc. 2N KOH	80	135	170	190

* The fact that 5 cc. of 30 per cent hydrogen peroxide is present in all these mixtures is omitted from this and subsequent tables for the sake of brevity.

TABLE 2.—*Experiment Demonstrating That It Is Not the Added Alkali Which Decomposes the Hydrogen Peroxide*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes			
		15 Min.	30 Min.	45 Min.	60 Min.
1	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH	90	140	165	180
2	44 cc. H_2O + 1 cc. 2N KOH	0	2	5	8

TABLE 3.—*Experiment of Table 1 Repeated with Use of Sodium Hydroxide Instead of Potassium Hydroxide to Show Relation of Concentration and Kind of Alkali to Yield of Gas (Oxygen)*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes			
		15 Min.	30 Min.	45 Min.	60 Min.
1	5 cc. fibrinate + 40 cc. H_2O (control)	0	0	2	2
2	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N NaOH	90	135	170	190
3	5 cc. fibrinate + 38 cc. H_2O + 2 cc. 2N NaOH	110	160	195	230
4	5 cc. fibrinate + 37 cc. H_2O + 3 cc. 2N NaOH	100	150	195	230

two alkalis are equally "strong," the sodium, in the lower concentrations at least, is not so effective an "activator" of the "ferment" as potassium.

The yield of oxygen is proportionate to the concentration of fibrinate. Table 4 illustrates the relation of the "concentration" of a "ferment" to the quantity of its activity.

The alkalinized fibrinate catalyzes an "infinite" amount of substrate. Tables 5 and 6 show how the same fibrinate mixture (with

TABLE 4.—*Effect of Decreasing the Concentration of the "Ferment" on the Yield of Gas*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes		
		30 Min.	45 Min.	60 Min.
1	10 cc. fibrinate + 34 cc. H_2O + 1 cc. 2N KOH.....	330	380	420
2	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH.....	200	230	260
3	1 cc. fibrinate + 43 cc. H_2O + 1 cc. 2N KOH.....	50	60	80

TABLE 5.—*Catalysis of an "Infinite" Amount of the Substrate by the Fibrinate, Sodium Hydroxide Being Used as the Alkalinizer*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes or Hours						
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.	24 Hr.
1	5 cc. fibrinate + 40 cc. H_2O (control)	0	0	5	18	25	...	100
2	5 cc. fibrinate + 38 cc. H_2O + 2 cc. 2N NaOH.....	105	155	190	220	245	...	540
3	5 cc. fibrinate + 36 cc. H_2O + 4 cc. 2N NaOH.....	120	190	240	280	310	...	515
4	5 cc. fibrinate + 34 cc. H_2O + 6 cc. 2N NaOH.....	120	205	260	300	325	...	480
5	5 cc. fibrinate + 32 cc. H_2O + 8 cc. 2N NaOH.....	130	200	248	280	305	...	465
	After 24 hours, 5 cc. more of 30 per cent H_2O_2 was added to each of these mixtures. The yields of gas were:							
		0	0	0	0	0	0	...
		45	65	95	120	145	165	...
		60	100	140	170	205	230	...
		65	115	165	195	220	250	...
		120	175	220	255	280	300	...
	When another 24 hours had elapsed, a third addition of 5 cc. of H_2O_2 was made. The evolution of gas was as follows:							
		0	0	0	0	0	0	...
		15	45	65	80	92	105	...
		50	80	110	145	170	195	...
		65	100	135	170	190	220	...
		110	180	230	265	300	320	...

TABLE 6.—*Catalysis of an "Infinite" Amount of the Substrate by the Fibrinate, Ammonium Hydroxide Being Used as the Alkalinizer*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes or Hours						
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.	24 Hr.
1	5 cc. fibrinate + 40 cc. H_2O (control)	0	5	10	20	25	30	...
2	5 cc. fibrinate + 38 cc. H_2O + 2 cc. 2N NH_4OH.....	65	100	115	130	145	160	...
3	5 cc. fibrinate + 36 cc. H_2O + 4 cc. 2N NH_4OH.....	70	90	115	135	150	165	...
4	5 cc. fibrinate + 34 cc. H_2O + 6 cc. 2N NH_4OH.....	80	120	140	170	180	195	...
5	5 cc. fibrinate + 32 cc. H_2O + 8 cc. 2N NH_4OH.....	90	140	160	190	205	225	...
	At the end of 24 hours a second 5 cc. of 30 per cent H_2O_2 was added to the aforementioned mixtures:							
		0	0	0	0	0	0	18
		2	7	18	20	25	30	380
		4	10	25	35	45	50	280
		8	25	40	55	68	80	500
		15	40	60	75	90	105	520
	And at the end of 48 hours, a third such addition was made:							
		0	0	0	0	0	0	...
		0	4	10	20	25	30	...
		2	18	25	40	50	60	...
		15	30	40	55	70	80	...
		10	30	50	65	80	98	...

sodium hydroxide used as the alkalinizing agent in the first and ammonium hydroxide in the second) repeats on successive days the same decomposition of hydrogen peroxide. Only these points need to be observed in regarding the tables: In absolute rate of decomposition the order of the active positive "ions" is $K > Na > NH_4$; also, the reaction mixtures "weaken," less oxygen being liberated on the second and third days of the experiment than on the first in the unit time.

Salts inhibit the activity of alkalinized fibrinate to varying degrees, dependent on their concentration and kind. The effects of increasing

TABLE 7.—*Inhibition of the Activity of an Alkalinized Fibrinate by a Salt (Potassium Chloride)*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes			
		15 Min.	30 Min.	45 Min.	60 Min.
1	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH (control)....	105	140	175	200
2	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH + 0.1491 KCl	90	135	165	185
3	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH + 0.2982 KCl	85	120	150	175
4	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH + 0.5964 KCl	72	110	140	160

TABLE 8.—*More "Poisonous" Effect of a Heavy Metal (Barium Chloride) on the Alkalinized Fibrinate*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes or Hours			
		30 Min.	45 Min.	60 Min.	16 Hr.
1	10 cc. fibrinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.208 Gm. $BaCl_2 2H_2O$.....	10	17	20	220
2	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH + 0.208 Gm. $BaCl_2 2H_2O$.....	5	8	10	160
3	1 cc. fibrinate + 44 cc. H_2O + 1 cc. 2N KOH + 0.208 Gm. $BaCl_2 2H_2O$.....	0	0	0	50

concentrations of a salt, chemically unreactive with any constituent of the ferment mixture, are illustrated in table 7. "Salting" obviously "preserves" the "catalase" mixture.

Heavier metals are more specifically "poisonous." The effects of barium are shown in table 8, the fibrinate mixtures being identical with those of table 4, but with barium chloride added in an amount not quite the equivalent of the 1 cc. of twice normal potassium hydroxide present in each. Any optical sign of precipitation of the barium as the hydroxide was absent.

Table 9 illustrates the effect on an active fibrinate-alkali mixture of the addition of chemical equivalents of a series of different chlorides.

The hardly perceptible suppressive effects of potassium become almost complete as calcium, mercury, magnesium and barium take its place.

These differences between the effects of different basic radicals hold also for the acid radicals. Tubes 2 and 3 of table 10 show how potassium chloride is less effective than potassium sulfate. In the remaining tubes the greatly suppressive activity of dipotassium sulfide is particu-

TABLE 9.—*Increasingly Suppressive Effects of Chemical Equivalents of a Series of Chlorides Added to the Fibrinate-Alkali Mixture*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes or Hours			
		30 Min.	45 Min.	60 Min.	16 Hr.
1	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH (control)	80	100	120	400
2	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH + 0.1492 KCl	80	100	115	390
3	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH + 0.111 $CaCl_2$	5	7	8	70
4	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH + 0.270 $HgCl_2$	0	0	2	50
5	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH + 0.095 $MgCl_2$	0	0	0	10
6	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH + 0.244 $BaCl_2 2H_2O$	0	0	0	140 (?)

TABLE 10.—*Differing Effects of a Series of Acid Radicals Added to the Fibrinate-Alkali Mixture*

Tube	Mixture	Cubic Centimeters of Gas Yielded in Given Number of Minutes					
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.
1	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH (control)	85	125	150	170	185	200
2	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH + 1 cc. 2N KCl	90	125	155	175	190	205
3	5 cc. fibrinate + 39 cc. H_2O + 1 cc. 2N KOH + 1 cc. 2N K_2SO_4	60	90	110	130	140	160
1	5 cc. K fibrinate + 39 cc. H_2O + 1 cc. 2N KOH (control)	65	95	120	140	160	170
2	5 cc. K fibrinate + 38 cc. H_2O + 1 cc. 2N KOH + 1 cc. 2N KNO_3	50	90	110	130	145	160
3	5 cc. K fibrinate + 38 cc. H_2O + 1 cc 2N KOH + 1 cc. 2N $KClO_3$	60	90	112	130	145	155
4	5 cc. K fibrinate + 38 cc. H_2O + 1 cc. 2N KOH + 1 cc. 2N K_2S	20	20	30	35	40	50
5	5 cc. K fibrinate + 38 cc. H_2O + 1 cc. 2N KOH + 1 cc. 2N phenol	150	220	295	350	400	430

larly noteworthy. Phenol, on the other hand, nearly doubles the rate of gas evolution.

In order not to lengthen this paper unduly, we merely state that the addition of the various potassium salts of the acetic series of acids to such mixtures as those named showed the lowermost members (formate through caproate) to inhibit as does ordinary sodium chloride. But by the time the caprate is reached, the decomposition of hydrogen peroxide is actually furthered (owing in our opinion to the fact that at the caprylate level these salts at the concentrations of water here

employed pass from solutions in the water to the hydrated soaps themselves, then in a physicochemical state identical with that of the protein ferment).[9]

V

We think that the experiments just detailed show how a protein, the fibrin from blood, is, by proper treatment with alkali, converted into a system which in every way behaves like the tissue extracts prepared for the study of catalase. Itself inactive, fibrin accelerates the decomposition of hydrogen peroxide as soon as it is properly alkalinized. In the terminology of ferments, a proferment is thus changed to a ferment. The material is active in the same low concentration in which recognized catalase is active, and it exhibits an optimal p_H. Acids retard or obliterate the decomposition effects of the fibrinate according to their concentration and kind just as they do this to standard catalase extracts.[10] The same fibrinate system produces an "infinite" amount of chemical change, corresponding in its behavior with any of the biochemist's "ferments." All salts reduce this activity, and where specific effects have been noted in the instance of specific salts in the study of catalase, parallelism is again complete. Even though large differences appear among the findings of different authors in this total field (due, no doubt, to the difference in the source of their catalase and in the history of their preparations), they agree in fundamentals. Thus ammonium always proves destructive at one end of a long series of salts carrying different basic radicals. The light metal salts show themselves least "poisonous"; they are followed in crescendo by the alkaline earths [11] and the heavier metals.[12] A similar harmony of effect on catalase and effect on our fibrinate exists for the acid radicals—the halogens being least inhibitory, with various "weak" organic acids occupying a middle zone, and the oxyacids proving in general most obviously "poisonous." [13]

We assume quite naturally that the described variations in the activity with which a protein decomposes hydrogen peroxide are associated with changes in its colloid chemical state as induced through

9. Fischer, M. H.: Soaps and Proteins: Their Colloid Chemistry in Theory and Practice, New York, John Wiley & Sons, Inc., 1921, p. 21.

10. Euler, H.: Beitr. z. chem. Phys. u. Path. **7**:1, 1905. Senter, G.: Ztschr. f. physiol. Chem. **44**:257, 1903.

11. Faitelowitz, A.: Zur Kenntnis Der Milchkatalyse Des H_2O_2, Dissert., Heidelberg, 1904 (not available in the original).

12. Euler, H.: Ark. f. kemi **2**:222, 1907. Favre, W.: Biochem. Ztschr. **33**:32, 1911.

13. For a summary of the voluminous and chiefly Scandinavian literature, see Oppenheimer, C.: Die Fermente, ed. 5, Leipzig, Georg Thieme, 1926, vol. 2, p. 1841.

changes in its surroundings (temperature, acidity or alkalinity, salt content, etc.). The protein must be made neither too soluble in water (as after treatment with ammonia) nor yet too little hydratable (as after treatment with a heavy metal). The greatest activity seems associated with the maximal degree of hydration *not* correlated with the maximal degree of dispersion (in "molecular solution" the protein again becomes inactive). This statement repeats in essence the view of the physical state of all ferments maintained by Fodor.[14] But our findings contribute further to what has long been a subject of debate. Reasoning from observed biochemical behavior, a majority has concluded that most ferments must be protein. Waentig and Gierisch[15] urged this for catalase specifically. To this we agree. As such, then, they become intimate blocks in the structure of living matter, in other words of that hydrated mass known as protoplasm.

SUMMARY

A potassium fibrinate is prepared from a shelf stock of blood fibrin and shown to behave like the catalase extracts of tissues or tissue juices. On the basis of qualitative and quantitative parallelisms a simple protein is thus stated to be the chemical equivalent of a ferment.

14. Fodor, A.: Das Fermentproblem, Dresden, Theodor Steinkopff, 1922, p. 172.
15. Waentig, P., and Gierisch, W.: Fermentforsch. **1**:165, 1916.

CASEIN AS CATALASE

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In a previous paper [1] it was shown how fibrin by proper chemical treatment may be made to assume all the activities of a "ferment." Through proper "alkalinization" this inactive protein from blood decomposed hydrogen peroxide as so much "catalase." These paragraphs detail how another globulin, the casein of milk, functions in identical fashion. The laboratory setup was as in the earlier experiments.

For "stock," 12.5 Gm. of casein (Harris) was soaked in 15 cc. of water overnight, and then 10 cc. of normal sodium hydroxide was added. The product was a thick paste. When diluted with water to 150 cc., it contained in the unit volume a quantity of protein approximately half of that of the same unit volume of the "standard" syrup of fibrin used in the studies cited.[1]

Sodium caseinate alone does not decompose hydrogen peroxide. Table 1 illustrates this fact as also the "activation" of the caseinate to "catalase" through the addition of alkali.

Table 2 exhibits an "optimal p_H" for the activity of the "ferment." It lies at a concentration of alkali less than that represented in mixture 5. The relation of the concentration of the "ferment" to the quantity of activity is illustrated in table 3.

Further proof that the decomposition of the hydrogen peroxide in these mixtures is more than the effect of their concentration of caseinate, p_H or "alkalinity" is furnished in table 4. Here equivalent concentrations of different alkalis were employed. Ammonium hydroxide is obviously less effective than potassium hydroxide or sodium hydroxide; while all three stand far above the hydroxides of calcium and barium, which, in fact, show themselves to be violently inhibitory.

How addition of increasing increments of a neutral salt (potassium chloride) to an active caseinate mixture of constant composition decreases its capacity to decompose hydrogen peroxide is apparent in table 5.

The effects of different acid radicals with constancy in the other components of the mixtures are shown in table 6.

From the Laboratory of Physiology, University of Cincinnati.

1. Fischer, M. H., and Suer, W. J.: Arch. Path., this issue, p. 815.

TABLE 1.—*Experiment Showing That Caseinate Acquires Catalytic Activity Only on Alkalinization*

Tube	Mixture*	Cubic Centimeters of Gas Evolved After Given Number of Minutes					
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.
1	10 cc. sodium caseinate + 35 cc. H_2O (control)	0	0	0	0	0	0
2	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N NaOH	55	90	120	150	170	195
3	10 cc. sodium caseinate + 33 cc. H_2O + 2 cc. 2N NaOH	73	135	185	230	270	300
4	10 cc. sodium caseinate + 32 cc. H_2O + 3 cc. 2N NaOH	100	170	227	275	315	350
5	10 cc. sodium caseinate + 31 cc. H_2O + 4 cc. 2N NaOH	190	210	270	320	360	390

Controls showed that the decomposition of hydrogen peroxide was not a matter merely of the addition of alkali. Pure alkali in the concentration represented in mixture 2 yielded the following amounts of gas:

	2	3	15	20	25	30

* The fact that each mixture includes 5 cc. of 30 per cent hydrogen peroxide is omitted from this and subsequent tables for the sake of brevity.

TABLE 2.—*Experiment Demonstrating the Relation of the Concentration of Alkali to the Catalytic Activity of the Caseinate-Alkali Mixture*

Tube	Mixture	Cubic Centimeters of Gas Evolved After Given Number of Minutes or Hours						
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.	24 Hr.
1	10 cc. sodium caseinate + 35 cc. H_2O (control)	0	0	0	0	0	0	0
2	10 cc. sodium caseinate + 33 cc. H_2O + 2 cc. 2N NaOH	25	90	120	150	180	200	510
3	10 cc. sodium caseinate + 31 cc. H_2O + 4 cc. 2N NaOH	42	150	210	255	290	325	510
4	10 cc. sodium caseinate + 29 cc. H_2O + 6 cc. 2N NaOH	100	180	240	275	310	335	520
5	10 cc. sodium caseinate + 27 cc. H_2O + 8 cc. 2N NaOH	125	190	235	265	285	300	500

TABLE 3.—*Experiment Demonstrating the Relation of the Concentration of Caseinate to the Catalytic Activity of the Caseinate-Alkali Mixture*

Tube	Mixture	Cubic Centimeters of Gas Evolved After Given Number of Minutes					
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.
1	20 cc. sodium caseinate + 24 cc. H_2O + 1 cc. 2N KOH	35	65	95	120	150	170
2	15 cc. sodium caseinate + 29 cc. H_2O + 1 cc. 2N KOH	30	60	85	105	130	150
3	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH	30	50	70	95	115	130
4	5 cc. sodium caseinate + 39 cc. H_2O + 1 cc. 2N KOH	30	50	70	85	100	112
5	2.5 cc. sodium caseinate + 41.5 cc. H_2O + 1 cc. 2N KOH	25	40	60	70	90	100

TABLE 4.—*Experiment Showing Differing Degrees of Catalytic Activity When Caseinate Is Alkalinized by Different Alkalis*

Tube	Mixture	Cubic Centimeters of Gas Evolved After Given Number of Minutes					
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.
1	10 cc. sodium caseinate + 35 cc. H_2O (control).....	0	0	0	0	0	0
2	10 cc. sodium caseinate + 35 cc. N/25 NH_4OH.....	6	25	40	55	65	75
3	10 cc. sodium caseinate + 35 cc. N/25 NaOH.....	12	35	52	65	80	100
4	10 cc. sodium caseinate + 35 cc. N/25 KOH.....	20	45	60	80	90	110
5	10 cc. sodium caseinate + 35 cc. N/25 $Ca(OH)_2$.....	0	0	0	0	0	0
6	10 cc. sodium caseinate + 35 cc. N/25 $Ba(OH)_2$.....	0	0	0	0	0	0

TABLE 5.—*Experiment Showing Increasingly Inhibitory Effect on Catalytic Activity of Alkalinized Caseinate with Additions of Increasing Amounts of a Neutral Salt*

Tube	Mixture	Cubic Centimeters of Gas Evolved After Given Number of Minutes						
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.	105 Min.
1	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH (control).....	10	35	55	70	90	100	115
2	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.1442 KCl.....	10	25	45	60	80	90	100
3	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.2884 KCl.....	10	25	40	55	70	82	95
4	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.4326 KCl.....	4	20	35	50	60	75	85
5	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.5768 KCl.....	2	10	30	40	50	60	70

TABLE 6.—*Differing Effects on Catalytic Activity of Alkalinized Caseinate with Addition of Different Acid Radcals*

Tube	Mixture	Cubic Centimeters of Gas Evolved After Given Number of Minutes					
		15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.
1	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH (control).....	22	45	70	87	105	120
2	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.0194 KSCN.....	32	60	80	100	115	130
3	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.0244 $KClO_3$.....	22	40	60	80	100	112
4	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.0202 KNO_3.....	22	40	60	78	100	112
5	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.013 KCN.....	10	25	42	57	70	85
1	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH (control).....	35	75	95	120	135	155
2	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.0332 KI.....	105	190	255	305	345	375
3	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.012 KH_2AsO_4.....	40	70	100	120	120	160
4	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.0196 $KC_2H_3O_2$.....	35	70	90	110	135	155
5	10 cc. sodium caseinate + 34 cc. H_2O + 1 cc. 2N KOH + 0.0162 KCNO.....	35	60	80	90	118	130

In order not to lengthen our protocols unduly we shall merely state that in oft-repeated series of experiments we found the inhibiting activity of different acid radicals or of different basic radicals to follow the general order discovered in the instance of fibrin functioning as catalase. For example, five mixtures containing different amounts of sodium caseinate (20 cc. to none) with constant amounts of potassium hydroxide and barium chloride yielded no gas whatsoever. The same proved true of calcium chloride. An exception was encountered in the instance of mercury: Its bichloride *increased* the decomposing effects of casein on hydrogen peroxide.

SUMMARY

Ordinary casein, itself inactive in the decomposition of hydrogen peroxide, may be made to function as catalase through the addition of alkali. But only the hydroxides of the lighter metals prove active in this regard. The conditions which make for the inhibition, or suppression, of an otherwise active mixture are identical with those which reduce catalase activity in biochemical extracts.

EXPERIMENTALLY INDUCED BENIGNANCY OF NEOPLASM

II. THE EFFECT OF TREATMENT WITH AN ESTROGEN AND OF CASTRATION OF THE HOST

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In a previous number of this journal Gardner, Smith, Strong and Allen [1] reported the production of sarcoma with an estrogen (theelin), administered subcutaneously. This interesting demonstration of carcinogenic effect suggests that an excess of estrogenic substance in the animal body favors malignant growth. It is possible, however, to demonstrate another effect of estrogenic substance: inhibition of the growth of sarcoma.

In previous papers from this laboratory dealing with susceptibility to inoculated sarcoma [2] the importance of the animal host was emphasized. It is clear that "malignancy" is not a property of the tumor alone. "Malignancy" is, rather, the reflection of the host's lack of immunity (or resistance) against neoplastic growth.[3] This resistance can be altered in experimental animals to varying degrees. Thus, in parallel experiments, inoculated pedigreed mice may show (*a*) no growth of tumors, (*b*) growth of "benign" tumors or (*c*) growth of "malignant" tumors.[2b] What factors influence this varying immunity?

One factor which may influence the degree of immunity is sex. Bittner [4] reported a difference in reaction to a transplanted tumor in males and females. Likewise, Andervont [5] in inoculation experiments

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1. Gardner, W. U.; Smith, G. M., Strong, L. C., and Allen, E.: Arch. Path. **21**:504, 1936.

2. (*a*) Salter, W. T., and Oster, R.: J. Clin. Investigation **15**:466, 1936. (*b*) Oster, R. H., and Salter, W. T.: Am. J. Cancer **32**:422, 1938.

3. Throughout this paper the term "immunity" is used in a specialized sense to indicate resistance against tumor growth on the part of the host.

4. Bittner, J. J.: Am. J. Cancer **16**:322, 1932.

5. Andervont, H. B.: Pub. Health Rep. **47**:1859, 1932.

with sarcoma 180 found that more than twice as many females became immune as males.[6] This finding suggests that androgenic and estrogenic hormones may influence the development of resistance against neoplasm. It is the purpose of this report to describe the effect of the administration of an estrogenic substance in excess and of castration on immunity to mouse sarcoma 180.

EXPERIMENTAL METHOD

Pedigreed mice (Bagg albino, strain A) were inoculated in the tail by the procedure of Andervont,[5] and the tumors were amputated after two to four weeks. The animals were then reinoculated in the groin. The growing tumors were measured with calipers in three diameters. These measurements were made twice weekly according to the procedures of Bischoff and Maxwell[7] and Schrek.[8] Control animals from the same generation of albino mice were inoculated in the groin without previous "immunization." These controls received tissue from the same tumor as the test animals. Each test animal was inoculated alternately with a control animal. The control tumors were measured simultaneously with the corresponding test tumors.

As a control strain, black mice of Bar Harbor strain C-57 were used. This strain becomes "immune" much less readily than the albino strain A.

The estrogen was administered according to the following schemes:

Scheme 1.—Test animals received daily subcutaneous injections of theelin[9] (estrone [3 hydroxy 17-keto-1,3,5-estratriene]) in peanut oil. The oil was injected into the back, far from the proposed site for inoculation of the tumor. After two weeks each animal was inoculated in the tail with tumor. Daily injections of the estrogen were continued. After two to four weeks the tail tumors were amputated. Each animal was then inoculated in the groin with the test tumor. Injections of the estrogen were continued without interruption. As soon as the groin tumors became palpable, measurements were begun.

"Immunity control" animals were treated in like manner except that peanut oil (0.1 cc.) was used in place of the solution of theelin.

"Virulence control" animals were completely untreated animals of the same generation. Each of these was inoculated in the groin with the test tumor. Thus the integrity of the tumor was assured.

Such controls were used in all subsequent schemes as needed.

Scheme 2.—Test animals received daily subcutaneous injections of the estrogen in peanut oil. After two weeks each was inoculated in the tail with tumor. Daily injections of the estrogen were continued. After two to three weeks the tail tumors were amputated. The injections of theelin were then discontinued. Each animal was inoculated in the groin with the test tumor. Measurements were made as outlined in scheme 1.

"Immunity control" animals and "virulence control" animals were maintained as in scheme 1.

Scheme 3.—Test animals received daily injections of theelin in peanut oil. After two weeks the injections were discontinued and each mouse was inoculated in the groin with the test tumor. No inoculation in the tail was made in this group.

6. This sex difference is not found in all strains of mice.
7. Bischoff, F., and Maxwell, L. C.: Am. J. Cancer **27**:87, 1936.
8. Schrek, R.: Am. J. Cancer **28**:345, 1936; Am. J. Path. **12**:525, 1936.
9. The theelin was contributed by Parke, Davis and Company.

Appropriate "immunity controls" and "virulence controls" were maintained.

Scheme 4.—Each animal was inoculated in the groin with the test tumor. Thereupon test animals received daily subcutaneous injections of theelin in peanut oil. Injections were continued throughout the period during which measurements were made.

Appropriate "virulence controls" were maintained simultaneously.

Scheme 5.—Test animals and "immunity controls" were inoculated in the tail with tumor. After two or three weeks the tail tumors were amputated. Immediately scheme 4 was started.

Scheme 6.—Castration was performed one month prior to inoculation of the groin with the test tumor. Afterward measurements of the inoculated tumors were made twice weekly.

Simultaneous "virulence controls" were maintained.

DOSAGE OF THE ESTROGEN

The daily dose of theelin varied from 50 to 200 international units. Other experiments performed in this laboratory on castrated animals indicate that 1 mouse unit of theelin (or estrone) is equivalent to approximately 1 international unit of crystalline estrone (or theelin). This is the amount required to produce estrus in the castrated animal. Accordingly, the doses used in these experiments must be regarded as more than ample.

EXPERIMENTAL DATA

The experimental results represent studies of 632 animals. The data were originally formulated as tumor growth curves, corresponding to about a month's time. Each tumor was ordinarily measured in three diameters twice a week. In order to condense the 9,000 values thus obtained, only mean diameters are presented in this report. Furthermore, in most instances only the mean diameters at maximal size (i. e., after three to four weeks' growth) are presented. The data are arranged in relation to certain specific questions.

1. *Does an excess of estrogen per se influence the growth of sarcoma 180?*

This problem was attacked according to scheme 4. The data are summarized in table 1.

Experiment 1.—Two groups of black adult males of the C-57 strain received different dosages of theelin. Group 1 received 50 international units per day; group 2, 200 units per day. Corresponding testicular weights are recorded as evidence of the effect of the estrogen. Appropriate "virulence controls" are shown.

Experiment 2.—A single group of black adult males received 200 units of theelin per day.

Experiment 3.—A single group of black adult females, C-57 strain, received 200 units of theelin per day.

Experiment 4.—A single group of albino adult males, strain A, received 100 units of theelin daily.

Experiment 5.—A single group of albino adult males received 200 units of theelin daily.

Experiment 6.—A single group of albino adult females, strain A, received 200 units of theelin daily.

From table 1 it will be seen that in experiments 1, 2 and 3 there was slight but definite inhibition of tumor growth. In fact, experiments

TABLE 1.—*Does Theelin per Se Influence Tumor Growth? (Scheme 4)*

	Controls for Virulence	Dose of Estrogen	
		50 I. U. Group 1	200 I. U. Group 2
Experiment 1: black males, C-57			
Number of animals	12	12	14
Mean tumor diameter at 3½ weeks, mm	16.9	11.0	9.0
Mean testicular weight, mg	84.4	72.7	56.9
Experiment 2: black males, C-57			
Number of animals	16	..	18
Mean tumor diameter at 3½ weeks, mm	17.2	....	12.5
Mean tumor diameter at 4 weeks, mm	20.3	....	15.0
Experiment 3: black females, C-57			
Number of animals	18	..	18
Mean tumor diameter at 3 weeks, mm	15.8	....	13.3
Mean tumor diameter at 4 weeks, mm	20.7	....	15.5
Summary of experiments 1, 2 and 3			
Number of animals	46	12	50
Mean tumor diameter at 3 weeks, mm	16.5	11.0	11.8
Experiment 4: albino males, strain A		100 I. U.	
Number of animals	14	14	
Mean tumor diameter at 3 weeks, mm	17.0	14.6	
Mean tumor diameter at 4 weeks, mm	20.3	20.5	
Experiment 5: albino males, strain A			
Number of animals	23	25	
Mean tumor diameter at 3 weeks, mm	13.0	11.0	
Mean tumor diameter at 4 weeks, mm	16.6	15.7	
Experiment 6: albino females, strain A			
Number of animals	24	18	
Mean tumor diameter at 3 weeks, mm	11.1	12.4	
Mean tumor diameter at 4 weeks, mm	15.3	17.5	
Summary of experiments 4, 5 and 6			
Number of animals	61	57	
Mean tumor diameter at 3 weeks, mm	13.4	12.4	
Mean tumor diameter at 4 weeks, mm	17.0	17.5	

TABLE 2.—*Effect of Experiment with Theelin on Body Weight*

	Virulence Controls	Dose of Estrogen	
		50 I. U.	200 I. U.
Male mice, C-57	10	10	10
Mean weight at start, Gm.	25.7	29.9	30.0
Mean weight after 3 weeks, Gm.	30.7	29.8	38.3

1 and 2 showed little or no overlapping between the controls and the treated groups, as indicated in figure 1. Furthermore, the inhibition paralleled testicular weight. It should be noted that our use of mean diameter as an index of growth tends to minimize differences in tumor growth. The differences shown in figure 1 would be more marked if volumes had been calculated from the three diameters, $V = 4/3\,\pi\,abc$, or

by the prolate-spheroid formula ($4/3\,\pi\,ab^2$), as used by Emge and Murphy.[10]

The animals were healthy mice of the black C-57 strain. Indeed, table 2 presents the gross weights of the estrogen-treated animals, which were not less than the control values. These weights indicate that cachexia was not a factor in the experiment.

At first sight one might be tempted to generalize and assert that the estrogen inhibited tumor growth directly. In the albino animals, however, practically no inhibiting effect was observed after the injection of the estrogen, as shown in table 1. In fact, the females of experiment 6

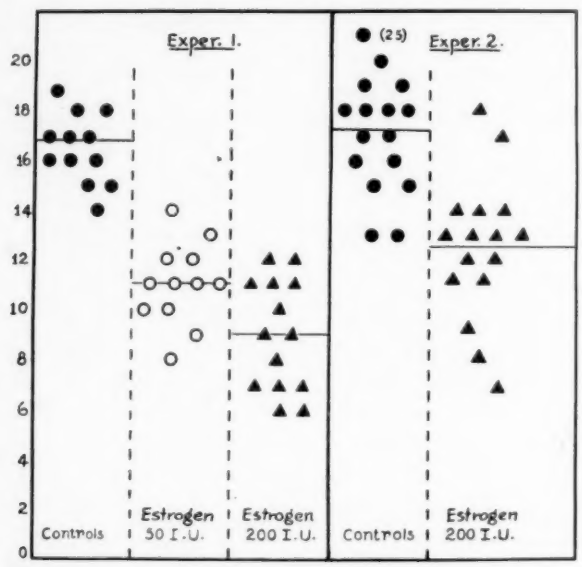

Fig. 1.—Effect of huge doses of theelin on size of tumors in mice. The values at the left represent the mean diameter of tumors in millimeters.

bore larger tumors than the controls. As will appear in table 3, further data substantiate this negative result.

In general, the answer to question 1 is apparently: No. The estrogen per se does not markedly inhibit the growth of sarcoma 180.

There remains to be explained the definite effect produced in the black animals of experiments 1, 2 and (possibly) 3. This result probably can be explained satisfactorily on the basis of an enhancement of the immune response, as described later under question 3. In short, the effect appears to be an indirect one.

10. Emge, L. A., and Murphy, K. M.: Am. J. Obst. & Gynec. **32**:593, 1936.

2. Does the estrogen (in excess) enhance the efficiency of "immunization"?

The problem was attacked according to schemes 1, 2 and 3. It can be considered in several phases:

(*a*) Preliminary treatment with the estrogen might make the animal more resistant to tumor growth (scheme 3).

(*b*) The estrogen might enhance the efficiency of the tail inoculation in producing "immunity" (scheme 2).

(*c*) A continuous excess of the estrogen (scheme 1) might be more effective than either (*a*) or (*b*) alone.

(*d*) In "immunized" animals the estrogen might enhance the previously induced inhibition of tumor growth (scheme 5).

As shown in table 3, possibility *a* is excluded. Experiment 1 on black males and experiment 2 on black females both gave negative results. It will be recalled, likewise, that table 1 shows no effect in albino animals, to which the estrogen was administered while test tumors were growing.

As to possibility *b*, little effect was demonstrated. Experiment 3 in black males and experiment 4 in black females showed no striking enhancement of immunity resulting from tail inoculation combined with simultaneous administration of theelin. Both of the groups subjected to tail inoculation had fewer "takes" and showed smaller tumors than the "virulence controls."

As to possibility *c*, the experimental data are definitely positive.

Experiments 5 a and 5 b in black males, C-57 strain, showed definitely fewer successful "takes" than their "immunity controls" or than the animals in experiments 3 and 4. It could also be shown that a definite effect on tumor size was obtained with high doses of theelin. In general, the average size of test tumors in animals receiving high doses of the estrogen was smaller than that of test tumors in animals receiving low doses. Of course, the test tumors of both groups were much smaller than those in the "virulence controls," i. e., the completely untreated. Furthermore, the relative diminution in size, compared with that in the "virulence controls," was more striking than that found in animals without tail inoculation (see *d*, below). The simultaneous shrinking of testicular weight is of interest as collateral evidence of estrogen effect.

In albino males, strain A, experiment 6 showed a striking complete resistance to the inoculated tumor. In short, no test tumors developed. It must be conceded that possibly the tumors used in experiments 6a and 6b were not particularly virulent.

Theelin, then, did definitely enhance the final outcome of immunization.

TABLE 3.—*Does Theelin Enhance Immunity?*

	Controls		Estrogen Treatment		Controls		Estrogen Treatment		Controls		Estrogen Treatment	
	Virulence	Immunity	Estrogen Alone	Tail Treatment Plus Estrogen	Virulence	Immunity	Estrogen Alone	Tail Treatment Plus Estrogen	Virulence	Immunity	Estrogen Alone	Tail Treatment Plus Estrogen
	Experiment 1, Scheme 3 Black Males, C-57				Experiment 2, Scheme 3 Black Females, C-57				Experiments 1 and 2 Combined			
Number of animals	12	..	12	.....	12	..	12	..	24	..	24	..
Mean tumor diameter at 3 wk., mm.	15.1	....	16.0	.....	14.8	..	15.2	..	15.0	..	15.6	..
Mean tumor diameter at 4 wk., mm.	20.3	....	20.7	.....	20.3	..	19.7	..	20.3	..	20.3	..
	Experiment 3, Scheme 2 Black Males, C-57				Experiment 4, Scheme 2 Black Females, C-57				Experiments 3 and 4 Combined			
Number of animals	10	15	..	15	10	15	..	15	20	30	..	30
Mean tumor diameter at 3 wk., mm.	15.1	10.2	....	10.7	14.8	11.4	..	11.0	15.0	10.8	..	10.9
Mean tumor diameter at 4 wk., mm.	20.3	13.9	....	13.4	20.3	15.7	..	15.1	20.3	14.8	..	14.3
Number of takes	9*	10	..	9	9*	9	..	8	18*	19	..	17
Percentage of takes	90*	66	..	60	90*	60	..	53	90*	63	..	57
	Experiment 5a, Scheme 1 Black Males, C-57				Experiment 5b, Scheme 1 Black Males, C-57				Experiments 5a and 5b Combined			
				50 I.U. 200 I.P.				50 I.U. 200 I.U.				50 I.U. 200 I.U.
Number of animals	6	15	..	12 12	6	9	..	12 12	12	24	..	24 24
Mean tumor diameter at 3½ wk., mm.	19.3	11.5	....	12.5 8.3	17.0	10.5	..	10.5 7	18.2	11.1	..	11.5 7.7
Mean testicular weight, mg.	90	..	..	57 28	87	..	..	64 34	87	..	..	61 31
Number of takes	6	6	..	5 2	6	6	..	4 3	12	12	..	9 5
Percentage of takes	100	40	..	42 17	100	66	..	33 25	100	50	..	38 21

	Experiment 6a, Scheme 1 Albino Males, Strain A				Experiment 6b, Scheme 1 Albino Males, Strain A				Experiments 6a and 6b Combined			
Number of animals	12	..	..	12	10	16	..	16	..	..	..	..
Mean tumor diameter at 3½ wk., mm.	16.3	....	....	0	17.6	8.9	..	0	..	..	..	..
Number of takes	10	..	..	0	10	6	..	0	..	..	..	..
Percentage of takes	100	..	..	0	100	37	..	0	..	..	..	..
Second Inoculation†												
Number of animals	10	..	..	..	10	10†	..	16†	52	16	..	28
Mean tumor diameter at 3½ wk., mm.	20.0	..	..	0	22	9.6	..	0	19	9.1	..	0
Number of takes	10	..	..	0	9*	2	..	0	51*	8	..	0
Percentage of takes	100	..	..	0	90*	20	..	0	98*	50	..	0
Third Inoculation†												
Number of animals	..	..	..	..	10	8†	..	16†	..	..	..	..
Mean tumor diameter at 3½ wk., mm.	..	..	..	..	19.8	0	..	0	..	..	..	..
Number of takes	..	..	..	..	10	0	..	0	..	..	..	..
Percentage of takes	..	..	..	..	100	0	..	0	..	..	..	..
Grand total of takes, percentage	..	..	..	..	97*	50	..	0	..	..	..	..

	Experiment 7, Scheme 5 Black Males, C-57				Experiment 8, Scheme 5 Black Females, C-57				Experiments 7, 8, 9a, 9b and 9c Combined			
Number of animals	10	15	11	14	9	9	12	12	51	52	53	70
Mean tumor diameter at 4 wk., mm.	20.3	12.5	15	9.3	20.3	12	13.6	10.1	20.0	13.1	19.0	8.7
Number of takes	9*	8	11	4	9	6	12	2	49*	31	53	21
Percentage of takes	90*	53	100	29	100	67	100	17	96*	60	100	30

	Experiment 9a, Scheme 5 Albino Males, Strain A				Experiment 9b, Scheme 5 Albino Males, Strain A				Experiment 9c, Scheme 5 Albino Males, Strain A			
Number of animals	10	..	10	13	12	12	10	13	10	16	10	18
Mean tumor diameter at 4 wk., mm.	19.6	..	18.7	6.1	17.4	10.2	18.2	7.3	22.1	16.5	21.1	10.1
Number of takes	10	..	10	5	11*	9	10	5	10	8	10	5
Percentage of takes	100	..	100	38	96*	75	100	38	100	50	100	28

* A second inoculation showed that susceptibility was really 100 per cent. In other words, the failure to take was due to a lapse in technic.
† Immune animals reinoculated. A new group of virulence controls was used.

As to possibility *d*, one would logically predict a positive effect; i. e., since possibility *c* minus possibilities *a* and *b* equals possibility *d*, the last should be the important feature. That this was indeed the case can be seen from table 3. Experiments 7 and 8 on black adult animals, strain C-57, indicated a definite increase in the number of completely immune animals. There was likewise a difference in size between those test tumors which did develop and the tumors in the "immunity controls." Of course, both were smaller than the tumors in the "virulence controls." The results agree rather well with those of experiment 5 as contrasted

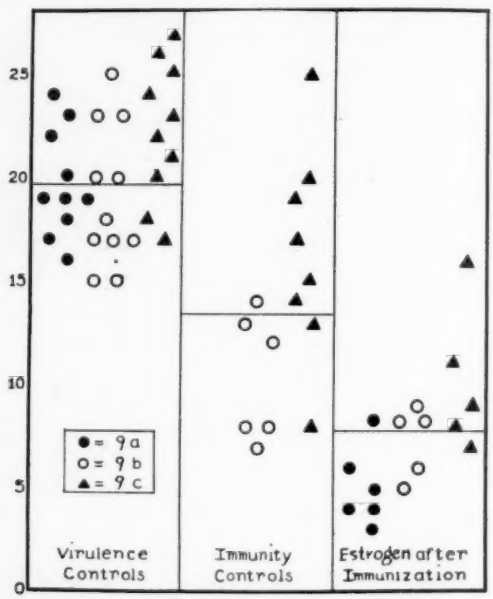

Fig. 2.—Effect of huge doses of theelin on size of tumors in tumor-immunized mice. The values at the left represent the mean diameter of tumors in millimeters.

with those of experiments 3 and 4. Experiments 9 a, b, c on albino adult males, strain A, gave similar results, as shown in figure 2.

In summary, the complete answer to question 2 is as follows: The estrogen does not increase markedly the efficiency of the immunizing procedure. After completion of the immunization, however, the estrogen markedly accentuates its effect.

Table 4 gives the results of statistical analysis of several experiments, appropriately combined. Only the immunized animals, with and without estrogen, have been compared. The probability that the difference reported is fortuitous is about 3 in 100 in individual groups. This figure neglects two other features which make for even less uncertainty, i. e.,

the considerable percentage of completely immune animals and the greater security of repeated observations.

It is known, in general, that some groin tumors provoke "immunity" against themselves as they grow. Thus Andervont [11] reported the frequent regression of sarcoma 37 after this tumor had attained a considerable size in strain M mice. Likewise sarcoma 180 grows and regresses in strain I mice.[4] No such regression of sarcoma 180 occurs ordinarily in the strains used in the present experiments. In partially immunized animals of albino strain A however, such regression does occur. Furthermore, as shown in table 1, experiments 1 to 3, the response of the black C-57 animals to theelin suggests that the estrogen enhanced a slight immune response which otherwise might have escaped notice.

TABLE 4.—*Statistical Analysis Indicating Possibility that Results After Injections of Theelin Were Mere Chance*

Experimental Combinations	Immune Controls		Animals Treated with Theelin After Immunization		n	s	t	P
	Number	Mean Diameter of Tumors, Mm.	Number	Mean Diameter of Tumors, Mm.				
I, 1 and 2.....	28	17.0	30	10.8	56	2.30	10.26	0.000
III, 5a and 5b.....	12	11.0	5	7.5	15	2.42	2.72	0.01(7)
III, 7 and 8.....	14	12.1	6	9.3	18	2.35	2.44	0.03
III, 9b and 9c.....	15	13.6	10	8.7	23	4.43	2.71	0.01(3)

n = degrees of freedom; t = difference of means divided by standard error of this difference, adjusted for n; P = probability of a difference as great or greater than that occurring by chance alone. (Fisher, R. A.: Statistical Methods for Research Workers, ed. 6, London, Oliver & Boyd, 1936, chap. 5. Mellor, J. W.: Higher Mathematics for Students of Physics and Chemistry, ed. 4, New York, Longmans, Green & Co., 1931, chap. 9.)

3. *What is the effect of castration on the "immune" response?*

This question is pertinent because gonadectomy in females reduces the natural supply of estrogenic substance.[12] One might expect, therefore, that the castrated female host would be less resistant to tumor growth.

The problem of castration was attacked according to scheme 6. Further study will be necessary in view of conflicting reports by Emge, Murphy and Schilling [13] and by Bischoff and Maxwell.[7] It is interesting, however, that our preliminary results are not inconsistent with the findings given earlier in this communication. In table 5 experiment 1 on black animals, strain C-57, indicates that tumors borne by the castrated female hosts were slightly larger than those in the controls. In experi-

11. Andervont, H. B.: Pub. Health Rep. **52**:1885, 1937.

12. Frank, R. T.; Goldberger, M. A., and Salmon, U. J.: Proc. Soc. Exper. Biol. & Med. **33**:615, 1936. Nathanson, I. T.: Unpublished data.

13. Emge, L. A.; Murphy, K. M., and Schilling, W.: Proc. Soc. Exper. Biol. & Med. **38**:21, 1938.

ment 2 there is perhaps an equivocal tendency in the same direction. The effect is not very striking, but the amounts of theelin involved are very much less than in the earlier experiments. In general, among the males there seems to be no striking difference from the controls, although a few test tumors were unusually large.

According to Murphy and Sturm,[14] the effect of castration varies with time. It may be that hypophysial hormones are involved in this phenomenon, as suggested by Druckrey.[15] Our few results are reported merely to show that the observed experimental effects of castration and of treatment with theelin, respectively, are not inconsistent.

LONGEVITY OF ANIMALS WITH ARTIFICIALLY BENIGN TUMORS

Sarcoma 180 is a highly malignant growth which kills the animal host soon after inoculation. For example, in a census of 131 inoculated control animals, all but a few were found to be dead eight weeks after

TABLE 5.—*Does Castration Influence Immunity?*

	Experiment	Controls C-57		Castrated Mice C-57	
		Males	Females	Males	Females
Number of animals	1	6	6	12	12
Mean tumor diameter at 3 weeks, mm		18.0	17.0	19.5	22.0
Mean tumor diameter at 4 weeks, mm		21.0	19.0	25.0	27.0
Average deviation at 4 weeks, mm		±2.7	±1.0	±3.3	±1.2
Number of animals	2	15	15	15	15
Mean tumor diameter at 3 weeks, mm		20.1	14.0	18.5	18.0
Mean tumor diameter at 4 weeks, mm		22.0	19.8	21.8	23.5
Average deviation at 4 weeks, mm		±1.7	±3.8	±2.2	±2.8

inoculation. In fact, 24 had died four weeks after inoculation and 89 by the sixth week. Thus 68 per cent were dead in a few weeks. A census of 29 partially immune animals in which small tumors grew showed a striking contrast; 2 had died at ten weeks, 1 at three months and 4 at five months. Thus after five months 76 per cent of the animals were living. In some, indeed, the tumors had regressed. This longevity of the host is striking evidence that artificially induced benignancy is real.

It should be noted that the huge doses of theelin used in our earlier experiments presumably influenced the pituitary, as reported by Cramer and Horning.[16] In interpreting the action of the estrogen, therefore, one must bear in mind the possibility of profound indirect effects. Provided that large doses of theelin inhibit pituitary function, a secondary inhibition of tumor growth might result. Such an effect was reported by Ball and Samuels[17] after hypophysectomy.

14. Murphy, J. B., and Sturm, E.: J. Exper. Med. **42**:155, 1925.
15. Druckrey, H.: Arch. f. exper. Path. u. Pharmakol. **181**:174, 1936.
16. Cramer, W., and Horning, E. S.: Lancet **1**:1056, 1936.
17. Ball, H. A., and Samuels, L. T.: Am. J. Cancer **32**:50, 1938.

COMMENT

These results are consistent with the concept [2] that "malignancy" is a graded condition depending on a balance between tumor and host. The reciprocal relationship between the neoplasm and the host's normal tissue can be altered by artificial variations in the immunization technic.[5] It can be altered likewise by endocrinologic changes in the host, as demonstrated in this report.

The literature contains many references to estrogen as an aggravator of malignant disease.[18] With few exceptions,[19] most of these citations have to do with neoplastic degeneration of primary or secondary sex organs. The observations reported herewith, therefore, are of special interest for two reasons: First, they picture estrogen as a motivator for benignancy. Second, they have to do with the suppression of a tumor of nondescript fibrous tissue, unrelated to sex functions.

The mechanism, however, whereby such suppression is produced remains obscure. It need not be a direct sterol effect.[20] It may well involve a rearrangement of the balance between various endocrine glands of the host. Indeed, Cramer and Horning [21] observed adrenal degeneration in pure strain mice subject to mammary cancer.

The literature also records other experiments designed to try the effect of androgenic and estrogenic substances on the growth of tumors. For example, Sugiura and Benedict [22] and Wiesner and Haddow [23] reported such studies. Some of these were surveyed by Bischoff and Maxwell.[7] The results of most of them were negative, in accord with our experiments on the effect of theelin per se. A few investigators, like Zondek, Zondek and Hartoch [24] and Nitta,[25] obtained positive results.

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18. Lacassagne, A.: Compt. rend. Acad. d. sc. **195**:630, 1932. Gardner, W. U.; Smith, G. M.; Allen, E., and Strong, L. C.: Arch. Path. **21**:265, 1936. Gardner, W. U.; Smith, G. M.; Strong, L. C., and Allen, E.: J. A. M. A. **107**:656, 1936.

19. Notably the work of Gardner, Smith, Strong and Allen.[1]

20. This statement does not mean that lipoids are unimportant in the production of cancer (cf. Claude, A.: Science **86**:294, 1937). Nor does it imply that immunity is not directly associated with the malignant process. It is interesting that, according to Haddow and his collaborators, carcinogenic agents produced inhibition of the growth of transplantable tumors, as well as of general body growth (Haddow, A., and Robinson, A. M.: Proc. Roy. Soc., London, s.B **122**: 442, 1937. Haddow, A.; Scott, C. M., and Scott, J. D.: ibid. **122**:477, 1937).

21. Cramer, W., and Horning, E. S.: Nature, London **139**:196, 1937; J. Path. & Bact. **44**:633, 1937.

22. Sugiura, K., and Benedict, S. R.: Am. J. Cancer **18**:583, 1933.

23. Wiesner, B. P., and Haddow, A.: Nature, London **132**:97, 1933.

24. Zondek, H.; Zondek, B., and Hartoch, W.: Klin. Wchnschr. **11**:1785, 1932.

25. Nitta, Y.: Jap. J. Obst. & Gynec. **19**:90, 1936; abstracted, Am. J. Cancer **31**:112, 1937.

The experiments presented herewith go far toward reconciling the apparent discrepancies between several investigators, some of whom relied on physiologic changes or doses of physiologic magnitude, while others (e. g., Zondek, Zondek and Hartoch) used large amounts of estrogen.

Among the negative effects reported for theelin are those of Emge, Murphy, and Schilling,[13] who studied the effect of this estrogen on transplantable mammary rat adenofibroma. Their findings in white rats were corroborated by our results in albino mice of strain A *when preliminary immunization was omitted.* Thus the apparent discrepancies in the literature can be explained on two grounds: that of dosage and that of immune reaction.

It should be emphasized that this work is not concerned with the *origin* of neoplasms. It has to do merely with their continued existence and growth. The animal host is regarded as an animated culture medium which may be made, to a varying degree, favorable or unfavorable to tumor growth. This concept was clearly established early in this century.[26]

SUMMARY

Pedigreed mice were artificially "immunized" against sarcoma 180 by preliminary inoculation in the tail. In such animals the "immunity" was enhanced by large doses of theelin, given while the test tumors were developing. The number of completely immune animals was increased. Furthermore, in nonimmune animals, strain C-57, the rate of growth of the implanted tumors was somewhat slowed by the administration of theelin while these test tumors were growing. In albino animals, strain A, however, the latter effect was not noted. The effect of the estrogen on the growth of the tumors, therefore, was probably not a direct inhibition on the tumors. The estrogen secondarily enhanced a primary inhibiting mechanism.

Castration of females possibly decreased resistance to tumor growth, but the effect was slight at best.

These results in toto suggest that the fate of an inoculated tumor is partly determined by the endocrinologic status of the host.

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26. Clowes, G. H. A.: Bull. Johns Hopkins Hosp. **16**:130, 1905. Bashford, E. F.; Murray, J. A., and Cramer, W.: Proc. Roy. Soc., London, s.B **79**:164. 1907. Lumsden, T.: Am. J. Cancer **15**:563, 1931; Lancet **2**:814, 1929.

EPITHELIAL FUNCTIONAL REJUVENATION OBSERVED IN THE MUCOUS CELLS OF THE GASTRO-INTESTINAL TRACT AND THE PARIETAL CELLS OF THE STOMACH

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Throughout the epithelial layer of the alimentary tract from the esophagus to the anus are found peculiar cells, scattered here and there among the other elements, which are known in histology as enterochromaffin or argentochrome cells. Their histogenesis, though a subject of research for more than fifty years, is still a matter of debate, and their physiologic role remains unknown. It is generally held that these cells are of entodermal origin. There are, however, investigators who claim ectodermal origin for them. Neither initial nor terminal stages of their histogenic cycle have been traced with certainty. There is no generally accepted concept concerning their function. The range of theories runs from restrained presumptions to fantastic assumptions. Some believe that they are externally secreting (exocrine) digestive glands. Others consider them as absorbing cells. On account of their topographic distribution and their specific reducing power they are regarded by some as glands of internal secretion. Their endocrine function is linked with the metabolism of carbone hydrate, the production of secretin, the secretion of epinephrine, the formation of antianemic factors and other processes. In the opinion of some investigators, argentochrome cells discharge their secretion neither into the lumen of the alimentary tract nor into the blood and lymph streams, and their secretion acts only locally on the nerves. The latter hypothetic action is designated as a neurocrine function. There is no better critical review of this controversial subject than that by Macklin and Macklin,[1] and further discussion of other theories may be omitted here. In closing their review on the nebulous condition of present day knowledge of the argentochrome cells Macklin and Macklin come to the conclusion that the most that can be said of the function of these cells is that it con-

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1. Macklin, C. C., and Macklin, M. T.: The Intestinal Epithelium, in Cowdry, E. V.: Special Cytology: The Form and Functions of the Cell in Health and Disease, ed. 2, New York, Paul B. Hoeber, Inc., 1932, vol. 1, p. 233.

stitutes an engaging problem, solution of which is a challenge to investigators.

From analysis of the vast literature on this subject it is apparent that progress in this field of research is hindered chiefly by limitations on the methods employed in the study of this problem. So far only histologic methods are available for the study of the physiologic and pathologic aspects of these cells, and the main and recommended methods of silver impregnation are complicated, time consuming and of doubtful specificity. Cumbersome and uncertain, they remain practically inapplicable to the study of argentaffin cells under various experimental conditions. Morphologic studies made with these methods cover the subject in a most exhaustive way, and nothing new can be expected from reexamination of this problem by these means.

In discussing the reasons for the limitations of the methods in use the following eight points of criticism are justifiable:

1. Trinitrophenol-formaldehyde solution (Bouin's picroformol fluid) is recommended as the fixative of choice, but repeated warnings are given to the effect that fixatives containing mercury bichloride or potassium bichromate are detrimental and should not be employed. For topographic and general cytologic studies Bouin's fluid is indeed valuable but is not generally recognized to be of value for fine histologic studies. It destroys much of the cell content and thus gives an incorrect picture of the cells. An ingredient of this fixative that is most injurious to the finer constituents of the cell is acetic acid. It is common knowledge among modern workers that a cell fixed in a solution containing acetic acid has a more "raked out" appearance than one fixed in a solution from which acetic acid has been omitted. This applies not only to cell granules but to the appearance of the ground cytoplasm, nucleoli and chromatin filaments (Lee [2]). By hemolyzing the erythrocytes acetic acid destroys their reducing power. Bouin's fluid is of no value for fine hematologic studies, and certainly it is not the fixative of choice for studies of intracellular structures.

2. Ammoniacal silver is considered to be the only proper solution for use in demonstrating silver-reducing granules. The ways of preparing this solution are not governed by exact standards of chemical procedure, and the time required for impregnation is long and beyond objective control. With the methods most commonly employed now, the step of silver impregnation alone requires from thirty-six to forty-eight hours.

3. The formation of metallic sols prepared with reduction methods, their dispersity, their stability and their precipitation depend on the type

2. Lee, A. B.: The Microtomist's Vade-Mecum: A Handbook of the Methods of Animal and Plant Microscopic Anatomy, ed. 10, Philadelphia, P. Blakiston's Son & Co., 1937.

of reducer employed. It might be expected, then, that by trying a new and more effective reducer different results could be obtained from histochemical impregnation.

4. In all present day methods, counterstaining is done after silvering, and it is questionable whether this way of counterstaining can be considered dependable in tracing various morphologic changes concomitant with the life cycle of the epithelial silver-reducing cell.

5. It is reasonable to expect that the initial formation, accumulation and disappearance of silver-reducing granules must be preceded or followed by detectable changes in the nuclei of corresponding cells. The methods in use offer no selective technic for the study of this aspect of the problem.

6. Simultaneous and practically unavoidable impregnation of various forms of wandering cells, macrophages, lymphocytes in transition, granular leukocytes and their forerunners is one of the most serious defects of methods employed now. This technical defect has served as a main source of confusion and has led a number of investigators to incorrect conclusions concerning the histogenesis and function of epithelial silver-reducing cells. It is obvious that the technical problem of tinctorial differentiation between epithelial silver-reducing cells and the various silver-reducing cells of mesodermal and ectodermal origin is of utmost importance.

7. It is claimed that the granules of Paneth cells are not impregnable with the silver methods commonly used. A reexamination of this question with the aid of a new technic is of considerable importance.

8. If specific diphenols are responsible for silver and chrome reduction, trials with the reduction of other metals are more than justifiable.

ADVANTAGEOUS FEATURES OF THE NEW TECHNIC

As a result of the investigations to be reported here, the objectionable points just discussed may be corrected by the use of a new technic based on entirely new principles. Introductory statements concerning the important features of the new methods, arranged in an order corresponding to that of the discussed eight points, are as follows:

1. In these newly devised methods the trinitrophenol-formaldehyde solution is replaced with a fixative introduced by Helly.

2. Ammoniacal silver is replaced with silver nitrate solution and the time of silver impregnation is reduced from forty hours to ten minutes.

3. A new and specially adjusted combination of hydrazine hydrate and water blue has been prepared and serves as the reducer of choice. Applied for five minutes, this solution acts simultaneously as a reducer and as a counterstain.

4. Counterstaining with safranin and with eosin is done before silvering, and the results offer new information pertaining to the life cycle of the silver-reducing cells.

5. With the combined eosin–potassium bichromate–water blue–silver technic, the silver-reducing cells show in their various phases of activity certain important nuclear changes which are not demonstrable with ordinary methods.

6. The rapid and easily controlled technic of silver impregnation and the use of certain chemicals, applied before silvering, have served to differentiate various tissue elements by disclosing either comparative degrees of silver-reducing power or loss of this power.

7. Paneth cells in a certain phase of their secretory activity show with the new technic granules which give a silver reduction reaction.

8. It has been found that with a modified technic the other metals (mercury, gold, tellurium and bismuth) may give reduction reactions similar to those obtained with silver. Their tinctorial effect is not as good as that obtained with silver, but their histochemical significance is identical.

PRINCIPLES UNDERLYING THE NEW METHODS

Before describing the new methods it is desirable to discuss (1) the principles underlying them, (2) the types of reagents employed and (3) the tinctorial and impregnation results obtained with each method. With all these newly devised methods the fixative employed is Helly's fluid. The essential prerequisite is that the tissues must be perfectly fresh. The tissues are run through alcohols and embedded in accordance with the chloroform-paraffin method.

The principle of the first and main silver method is based on the reduction of silver nitrate with a hydrazine hydrate–water blue mixture. When the hydrazine hydrate ($H_2N\text{-}NH_2$) is added to the solution of water blue, there is formed immediately a yellowish leukocompound. Acting as a reducer, this leukocompound, on reoxidation and change of p_H, gives also an excellent effect of counterstaining. Hydrazine hydrate alone, applied in equivalent dilution, fails to give reductions similar to those obtained with the leukocompound. A leukocompound formed on heating the solution of water blue with zinc dust and applied to sections after silvering gives only faint traces of reduction. It is apparent, then, that the selective efficiency of the new reducer depends on a fitting combination in one solution of hydrazine hydrate and water blue. With this reducer, a solution of silver nitrate as weak as 0.04 per cent applied for ten minutes only is sufficient to impregnate epithelial silver-reducing granules. An important step, without which the methods described fail to work, is a thorough treatment of the sections with aqueous solution of iodine, and this must be done before silvering.

The main silver method impregnates in a specific way the granules of certain epithelial and mucous cells, the nuclei of certain mucous cells, the granules of certain parietal cells of the stomach, certain granules of Paneth cells, macrophages, certain leukocytes and the fine reticulum. Helly's fixative preserves excellently all the blood elements, and the method just described impregnates also the erythrocytes with an effect that is equal to that obtained with any good peroxidase reaction.

In the first variant of the main method sections are stained first with safranin. Then they are treated with silver nitrate solution and reduced with the hydrazine hydrate–water blue leukocompound. The unusual value of this method is that it stains the mucus of the goblet cells selectively and with tinctorial differentiations corresponding to the various phases of their life cycle. In line with the tinctorial differentiation of mucus it depicts also corresponding stages of cellular transformations which are associated with the appearance of the silver-reducing granules in the mucous cells.

In the second variant of the main method sections are stained first with the potassium bichromate–eosin–ammonium hydroxide mixture. Then they are treated with silver nitrate solution and reduced with the hydrazine hydrate–water blue leukocompound. Though both the dyes employed (eosin and water blue) are acid, the tinctorial effect obtained is such that eosin acts as a nuclear dye and stains only nuclei of certain mucous cells. The nuclei of other mucous cells either are impregnable with silver or appear violet blue, blue and light greenish blue. Comparing the tinctorial effect of this method with that of the main silver method, one perceives that the nuclear eosinophilia and the nuclear silver-reducing power shown by certain types of mucous cells are similar in their biologic significance. While the nuclei are silver reducing with the main method, with the second variant of that method the nuclei of the same types of mucous cells appear eosinophilic or silver reducing. In other words, these two methods are reciprocal. With this second variant the granules of eosinophilic leukocytes stain a deep red, and this facilitates cellular differentiation, which is rather difficult with the main silver method, as the latter impregnates the granules of these cells and the granules of true epithelial cells with almost equal intensity. The nuclei of active histiocytes, especially of those engaged in the formation of reticulum, are stained red with this variant method, while their fine protoplasmic processes appear black. This method offers also additional facilities for differential studies of the transformation forms derived from free and fixed elements of the mesenchyme.

The results obtained indicate that the common affinity of certain tissue elements for silver can be dissociated by a combination of various tinctorial and chemical factors. Of chemical factors, the p_H of the water used for washing sections before the sliver bath and the strength of the

silver nitrate are of essential importance. Washing the sections before silvering in water of above p_H 7 intensifies or increases the impregnation phenomenon, while washing in water of below p_H 7 weakens it. If the wash water used before silvering is of p_H 7 and the time of silver impregnation is the same, the comparative degree of the silver-reducing power of each tissue element can be followed up by modifying the strength of the silver nitrate solution used. Thus, on decreasing the strength of the silver nitrate solution from 4 per cent to 0.04 per cent, the combination of the three methods described facilitates to a great extent the matter of cellular differentiation. In addition to this, the following simple test has been found of service: If sections are treated first with 30 per cent hydrogen dioxide in an alkaline medium for fifteen minutes and then the main silver method is applied, some epithelial cells lose and some lessen their argentaffinity, while the argentaffinity of mesenchymal elements remains intact.

Mercurous nitrate ($HgNO_3H_2O$) in a 2 per cent aqueous solution applied for three to five minutes and reduced with the hydrazine hydrate–water blue leukocompound gives a reduction reaction which is equivalent to that obtained with silver. Similar reduction reactions are obtained on treating sections with this mercurous nitrate solution and reducing it with a 4 per cent aqueous solution of stannous chloride ($SnCl_2$ $2H_2O$) or with ammonium sulfide (light hydrosulfide solution).

Sections treated with gold chloride solution and reduced with hydrazine hydrate leukocompound fail to show a selective gold reduction reaction. If, however, sections are treated first with 1 per cent potassium tellurite or with a 2 per cent bismuth nitrate-d-mannitol solution or with 50 per cent formic acid and are then reduced with the hydrazine hydrate–water blue leukocompound and toned afterward with gold chloride, reduction reactions take place in the cells which corresponds to those of epithelial argentaffin cells.

MATERIAL EXAMINED AND SCOPE OF EXPERIMENTATION

Simple in technic and selective in results, the methods described offer a new opportunity for studies of the morphologic and functional aspects of the silver-reducing cells of the gastrointestinal tract. In the present studies only rabbits and fresh human surgical material were used. The human surgical material examined included a large variety of inflammatory and neoplastic processes. The specificity and selective property of these newly devised methods depend essentially on the freshness of the tissues, and for this reason no human postmortem material was used in these studies. In this work entire attention was concentrated on subjects pertaining to the histophysiologic aspects of the epithelial silver-reducing cells, and material obtained from normal rabbits, and rabbits used for experiments formed the foundation of the investigation.

The purpose of these experiments was to investigate the response of the intestinal epithelial silver-reducing cells after local and general application of various chemical substances. This response was studied in conjunction with concomitant changes in other constituents of the mucosa. The same material was also used for studies of the terminal vascular system of the gastrointestinal tract,[3] and for this reason extremely detailed studies of the entire wall were made in every instance. The stomach, small intestine, appendix and sigmoid (including the rectum) were studied in rabbits. Control tissues were taken from normal fasting animals kept on full vitamin diet, from animals at different stages of digestion and from animals kept on different types of diet.

TECHNIC OF EXPERIMENTS ON RABBITS TO ASCERTAIN EFFECT OF APPLICATION OF CHEMICAL SUBSTANCES TO GASTROINTESTINAL MUCOSA

The local effects of various chemical substances were studied in the following way:

The animal was kept fasting for twenty-four hours and was then anesthetized with ether. The abdomen was opened, and on both sides portions (loops) of the intestine were ligated with the least handling possible and away from blood vessels. The chemical substance was warmed to body temperature and introduced into the lumen with the aid of a syringe needle (G-24) and through a spot free from visible capillaries. Into an adjacent loop, made in a similar manner, as a control, was injected physiologic solution of sodium chloride. The needle was withdrawn without signs of hemorrhage or return leaking. In some comparative experiments two or three isolated loops were made in the same segment of the intestine, and into each loop a different substance was introduced. The abdomen was then closed with sutures and the animal, left alone without anesthetic, was kept alive for a period varying from ten minutes to one hour. On completion of the experiment the rabbit was disposed of either by an injection of air into the ear vein or by a blow over the neck. The abdomen was then reopened, the mesenteric blood vessels supplying the experimental and the control portions of the intestine were ligated, and the entire segment of intestine was removed gently and without loss of blood. In each instance the segment removed contained an adjacent part of the intestine into which the chemical substance had not been injected as a control.

The whole tissue was then placed in Helly's fixative for fifteen minutes. Each individual loop, including the control loop, was cut across into small segments; the lumen of each segment was washed out with Helly's fixative, and the trimmed segments were placed in fresh Helly's fixative for twenty-four hours. Control tissues also were fixed in this way. After being removed from the abdominal cavity, the intestinal loops were immediately cut across into small segments, and the content of each segment was washed out with 10 per cent formaldehyde and placed in Helly's fluid for twenty-four hours. Certain parts of the intestine, even after being removed from the abdominal cavity, remained sensitive to handling and to thermal shock. This necessitated controls with fixation performed in situ.

3. Popoff, N. W.: Arch. Path., to be published.

The latter was done by disposing of the animals in the manner already described and filling the abdominal cavity with the Helly fixative, which had been warmed to body temperature and introduced without touching the experimental and control portions of the intestine.

Experiments also were made with the object of studying the effects on the metallic reduction reaction itself of a delay in fixation of from ten minutes to four hours. These experiments were helpful in offering an opportunity for further study of the phenomenon of Mingazzini. The entire work reported here was based on examination of tissues with the aid of serial sections.

The following fifteen chemical substances were investigated with regard to their respective local effects on silver-reducing and other cells of the intestinal tract:

1. Epinephrine in solution (1:3,000 to 1:1,000)
2. Atropine (0.02 mg. dissolved in 5 cc. of distilled water)
3. Benzyl benzoate in solution (benzyl benzoate, 20 per cent; alcohol, 75 per cent), with 75 per cent and 40 per cent alcohol as parallel controls
4. Physostigmine (1 mg. dissolved in 5 cc. of distilled water)
5. Dextrose in solution (1:10)
6. Histamine in solution (1:10,000 to 1:1,000)
7. Lactic acid in solution (1:20)
8. Aqueous solution of iodine (in strength originally employed by Gram)
9. Magnesium sulfate in solution (1:10 and 1:5)
10. Sodium carbonate in solution (1:20)
11. Volatile oil of mustard U. S. P. (3 per cent in olive oil)
12. Ox bile (10 per cent dried *Bacto* ox bile in distilled water)
13. Pilocarpine (0.015 mg. dissolved in 5 cc. of distilled water)
14. Sodium nitrite (0.003 mg. dissolved in 5 cc. of distilled water)
15. Silver nitrate in solution (1:20)

In the study of general or indirect effects, the following substances, administered subcutaneously, were used:

1. Epinephrine, 0.5 mg.
2. Histamine, from 0.05 to 1 mg.
3. Pilocarpine, 0.5 and 1 mg.

Animals were disposed of at different intervals after injection, and tissues were taken care of in accordance with the technic already described.

HISTOLOGIC TECHNIC

Tissues were fixed in Helly's fluid for twenty-four hours, washed in running water for twenty-four hours, dehydrated in ascending alcohols (the time not exceeding thirty-six hours altogether), placed in chloroform for twelve hours and in chloroform saturated with paraffin twelve hours, passed through three changes (five hours each) of pure paraffin without beeswax and then embedded in the usual way.

Serial sections were attached to the slides with the aid of Masson's gelatin–dilute formaldehyde method (Masson [4]). To remove the paraffin, sections were run through three changes of xylene. On being taken out of the last change of xylene, the slides were plunged into 0.2 per cent pyroxylin in alcohol-ether for one to two minutes; they were removed and the excess of pyroxylin solution was drained off quickly, after which they were placed in 80 per cent alcohol. Devised by Regaud, this method of pyroxylinization assured perfect attachment of serial sections, which is essential in work with metallic impregnation. From the 80 per cent alcohol the sections were transferred to distilled water (p_H 7) for fifteen minutes (three changes of five minutes each), placed in Weigert's aqueous solution of iodine for twenty-five minutes, run through distilled water (p_H 7) for three minutes, 95 per cent alcohol for five minutes and distilled water (p_H 7) for three minutes, placed in 4 per cent sodium thiosulfate for ten minutes and washed in three changes of distilled water (p_H 7) for fifteen minutes altogether. The sections were then ready for staining and silver reduction.

The first or main method requires the following six reagents: (1) distilled water of p_H 7, (2) distilled water of p_H 6, (3) 4 per cent silver nitrate solution, (4) reducer, (5) 1 per cent acetic acid, freshly prepared each time to avoid contamination with molds, and (6) 4 per cent sodium thiosulfate.

The silver nitrate solution is made of Merck's blue label silver nitrate dissolved in freshly distilled water. The distilled water used is obtained with the aid of an all metal distiller, and no interference with metallic impregnation reactions is noticed. The stock solution of silver nitrate is stored in a perfectly clean bottle with a glass stopper and kept in a dark place. The working solution is made each time by filtering the required amount into a perfectly clean brown glass dropping bottle.

The reducer is prepared by adding to 10 cc. of aqueous 2 per cent water blue (*Wasserblau*-6B Extra P-Holborn) 10 drops of hydrazine hydrate (Eastman Kodak-P902 42 per cent in water). As the hydrazine hydrate is added drop by drop and the mixture shaken, the blue color of the water blue solution is rapidly changed to a light port wine color. This leukocompound is filtered into a perfectly clean brown glass dropping bottle, and if kept in a dark place and tightly closed with a well grounded glass stopper it does not deteriorate for quite a long time. In work reported in this paper a freshly prepared reducer was used each time.

The technic being simple and rapid, all procedures of the combined staining and reduction are carried out on the slide at room temperature, the only precaution being to avoid exposure to direct light.

Method 1.—This is the silver–water blue method. The steps are as follows:

1. Use a dropping bottle flood section prepared as directed in the foregoing text with 4 per cent silver nitrate and leave it on the slide for ten minutes.

2. Drain off the excess of silver solution and blot the slide with a pad of filter paper. When blotting the next slide, use a *clean space* on the filter pad.

3. Without letting the section become dry, flood it quickly with hydrazine hydrate–water blue leukocompound and leave this applied for five minutes. The section turns rusty brown and is covered with a multitude of minute bubbles.

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4. Masson, P.: Diagnostics de laboratoire: Tumeurs—diagnostics histologiques, Paris, A. Maloine & Fils, 1923.

4. Wash the section by gently pouring distilled water of p_H 7 from a beaker, and after washing blow gently over the surface of the slide. Repeat this procedure quickly three times. The section turns yellowish green.

5. Place the slide in 1 per cent acetic acid, moving it up and down a few times, and leave it in the acid for five minutes. Here the section turns blue.

6. Place the slide in distilled water of p_H 7 and wash it in three changes of distilled water of p_H 7 for three minutes altogether.

7. Flood the section with 4 per cent sodium thiosulfate for four minutes.

8. Rinse and then place the slide in distilled water of p_H 6 for three minutes, moving it up and down a few times. During this time the excess of blue is removed. Then transfer the slide into a fresh change of distilled water of p_H 6 for seven minutes.

9. Dehydrate the section in 95 per cent alcohol for thirty seconds and follow with two changes of absolute alcohol of one minute each.

10. Prepare with xylene and balsam in the usual way.

Six slides can be handled easily at one time, the entire procedure taking forty-five minutes. Up to procedure 6 it is important each time to wipe off the end of the slide which is handled with metal forceps or to have the ends of the metal forceps well coated with paraffin. This procedure is to be used in all methods described.

Method 2.—This is the safranin–silver–water blue method.

1. After deparaffinization and treatment with aqueous solution of iodine, wash the sections as in the first method, with three changes of distilled water of p_H 7 for fifteen minutes.

2. Flood the sections with alcoholic safranin solution for ten minutes. This stain is made by diluting saturated alcohol-soluble safranin (Hollborn) with an equal part of distilled water of p_H 7. A 25 per cent aqueous solution of heat-saturated water-soluble safranin (Hollborn) may be used. Distilled water of p_H 7 is used in making safranin solutions.

3. After the treatment with safranin, wash the sections in three changes of distilled water of p_H 7 for three to five minutes altogether and then follow the technic of the first method from step 1 with only two modifications: (*a*) Instead of 4 per cent silver nitrate use 2 per cent silver nitrate, and (*b*) after slides are placed in 1 per cent acetic acid, keep them there for three minutes (here they give off the excess of safranin). Then transfer them into a fresh change of 1 per cent acetic acid and leave them in this for an additional three minutes.

Method 3.—This is the potassium bichromate–eosin–silver–water blue method.

1. Prepare sections for staining as described in the foregoing text.

2. Stain sections with the potassium bichromate–eosin–ammonium hydroxide mixture for twenty minutes. To make this mixture add to 7 cc. of 6 per cent aqueous potassium bichromate 3 cc. of 1 per cent yellowish water-soluble eosin (Hollborn), and to this mixture add 1 drop of ammonium hydroxide.

3. Using distilled water of p_H 7, rinse the sections and wash them with two changes of water for two minutes altogether.

4. Proceed now from step 1 of the first method. If the potassium bichromate–eosin mixture employed in this method is made without adding ammonium hydroxide, is filtered and applied, it gives somewhat different tinctorial and impregnation results, which are very instructive for comparative study.

The reagents used and the basic principles of the seven variants of the reduction tests made with mercury, gold, tellurium and bismuth have been discussed previously in this paper. Whether impregnation in these tests is combined with staining or is employed alone, the pretreatment with aqueous solution of iodine is essential.

RESULTS FROM STUDY OF THE SIGMOID

In describing the results of these investigations each anatomic part of the alimentary tract is considered separately. In this report the sigmoid is chosen first for the following reasons: (1) Compared with other parts of the alimentary tract, it has the simplest histologic structure and (2) its secretory product comes from one cellular source and does not contain significant amounts of enzymes. The sigmoid is devoid of villi; its glandular coat is lined with simple columnar epithelium, rich in goblet cells, and, as a rule, no cells of Paneth are found. Lymphoid follicles are scattered singly, and this permits studies on selected parts of the intestines devoid of this tissue-complicating structure.

The sigmoid of a full grown young rabbit that has been kept fasting for twenty-four hours is taken as the standard normal control. The results obtained with the newly developed methods are constant and uniform. The commonly observed type of silver-reducing cell in the sigmoid is that which is described in textbooks of histology as an argentaffin cell. With its broad base adhering closely to the basement membrane, this cell has the form of a conical flask with a cytoplasmic continuation directed toward the lumen. In certain instances only this type of silver-reducing cell is found (fig. 1).[5] It is the easiest type of cell to demonstrate, as it gives positive impregnation even with a very weak solution of silver nitrate (0.04 per cent applied for ten minutes). Figure 1 shows that these cells are equally numerous in the tip and at the bottom of a fold.

Neither the type nor the number of argentaffin cells is the same everywhere. Some sections show a considerable number of these cells scattered here and there, and in others none are found. On examining a vast variety of serial sections one notes that coincident with impregnation of granules in argentaffin cells there is silver impregnation of granules in certain mucous cells (fig. 2*A*). On applying a gradually decreasing concentration of silver nitrate (4 to 0.04 per cent) one finds that the impregnation shown by the granules of these mucous cells cannot be attributed to a fault in technic. In some mucous cells the granules are small and few (fig. 2*A*, right side of the field). In other cells they are large, numerous and segregated chiefly in the perinuclear zone (fig. 2*A*, central and left glands). The nuclei in these cells are pushed to the base, their structure appears indistinct, and they also show the

5. The photomicrographs were prepared by Mr. M. C. Orser, of the school of medicine of the University of Rochester.

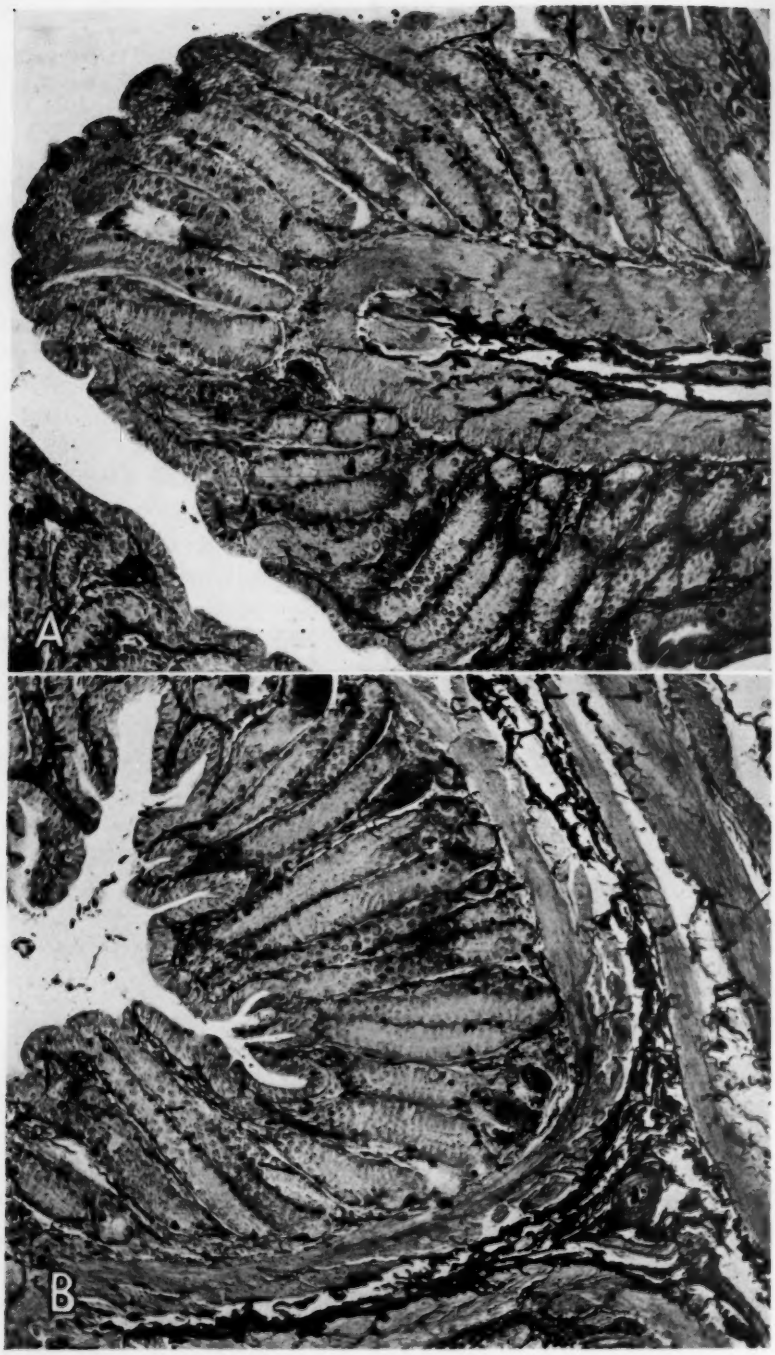

Fig. 1.—Two fields of the same section, showing argentaffin cells equally numerous in the tip and in the bottom of a fold of the sigmoid (× 100).

silver-reducing property (fig. 2 *A*). These cytoplasmic and nuclear phenomena are observed repeatedly and in a vast variety of materials. With the second, or safranin–silver–water blue, method, some of the mucous cells stain a deep safranin–Van Dyke brown, some pure deep safranin, some light safranin; some take only water blue, and certain cells do not take any stain, remaining, so to speak, chromophobe (fig. 3 *C* and 11 *B*). The silver reduction retains a selectiveness similar to that obtained with the first method. These observations indicate that there is a definite interrelationship between tinctorial types of mucous content and the positive or the negative reduction shown by the corresponding cells. The first appearance and gradual increase in cytoplasmic and nuclear power to reduce silver takes place side by side with a tinctorial change of mucous content from pure safranin to mixed safranin–Van Dyke brown.

The nuclear changes just described are of considerable importance. The information furnished with the two methods applied is insufficient, however, to enable one to draw any conclusions as to the significance of the nuclear changes observed. With the third, or potassium bichromate-eosin–silver–water blue, method, some nuclei take water blue, some show affinity for eosin, and some show positive reduction of silver. It is in those mucous cells which show nuclear affinity for eosin or silver that cytoplasmic silver-reducing granules are found (fig. 11 *C*).

It appears, then, that the structure of the normal sigmoid as seen with the new methods is far from simple. There can be no doubt that under normal circumstances the mucous cell passes repeatedly through successive phases of secretory activity. As the result of slow and incomplete evacuation the fully developed mucous cell, which will be designated as cell type 1, undergoes a change in appearance and is transformed into cell type 1-a. When evacuation is rapid and complete, type 1 is transformed into a slender, darkly staining cell, or type 1-b. In both instances the cell after evacuating its contents returns to the original state or, so to speak, becomes type 1. These repeated and reversive changes represent the normal functional cycle of the fully developed mucous cell (fig. 4 *I*).

In tracing these reversive morphologic changes one sees that in some instances certain mucous cells fail to empty their content in the usual fashion. The retained mucous content acquires gradually the appearance of inspissated mucus, and its tinctorial property is changed. Instead of pure safranin, it stains safranin-orange or safranin-brown (fig.11 *B*). The nucleus begins to take eosin and later on acquires the silver-reducing property. Side by side with this there appears in the cytoplasm a granular silver-reducing substance. The latter is found more abundantly in the basal and perinuclear zones of the cell. This cell is designated as type 1-c (fig. 2 *A*). As a further step in cytomorphosis, type 1-c is

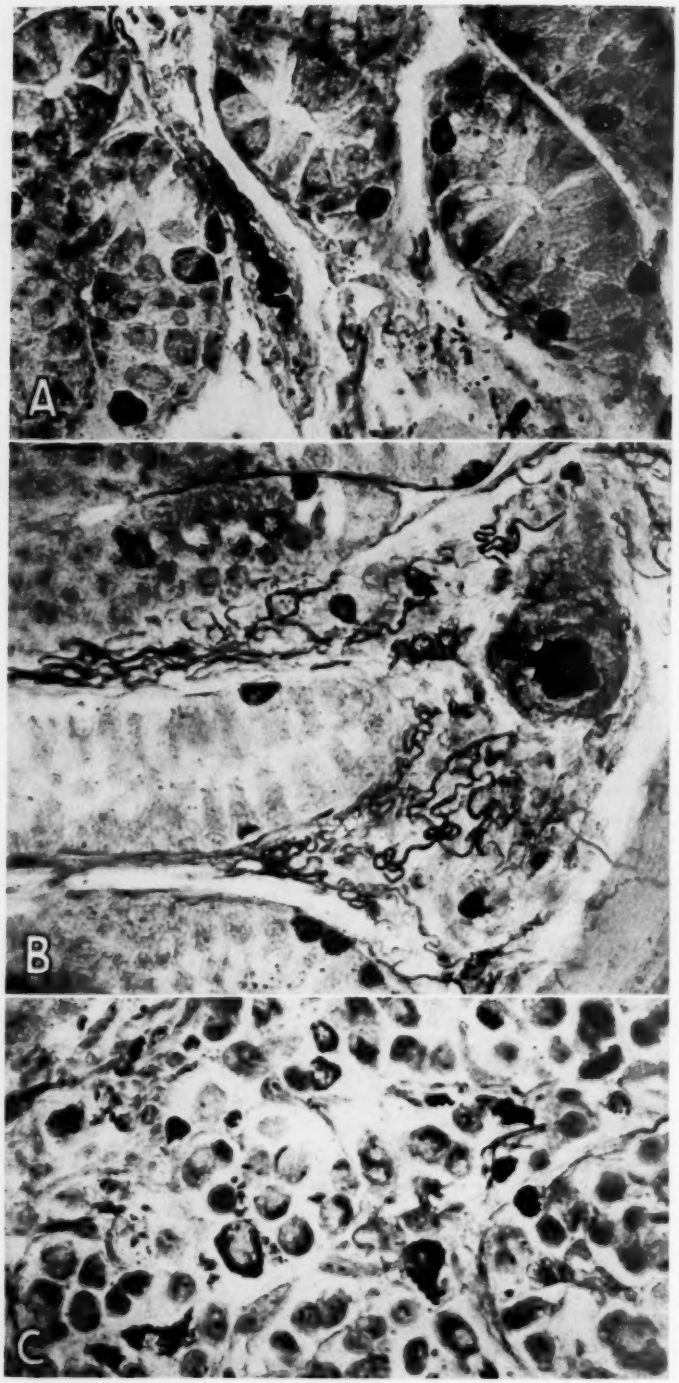

Fig. 2.—*A*, first stage, and *B*, second stage, in transformation of goblet cells of the sigmoid into argentaffin cells. In both *A* and *B* erythrocytes show selective reduction with silver. *B* shows extraglandular argentaffin cells of a mesenchymal nature; *C*, metastatic focus of gelatinous carcinoma with typical argentaffin cells (× 430).

altered in the following ways: (1) It shrinks, and the cytoplasmic connection with the lumen of the intestine becomes progressively narrower; (2) it loses its property of staining with safranin; (3) its silver-reducing substance becomes more condensed and prominent; (4) the entire cell sinks gradually toward the basement membrane, and (5) the nucleus appears pushed to the basal part of the cell. It resembles now a typical argentaffin cell and is designated as type 2 (fig. 2 *A*, upper cell in central gland, and fig. 4). On further recession of type 2 toward the basement membrane, the cytoplasmic connection with the lumen of the intestine becomes narrower and more pointed, and finally the cell retracts to such an extent that connection with the lumen no longer exists. It is now discoid, the inner convexity, facing the basement membrane, being flatter and broader. The nucleus of this cell loses its affinity for eosin and shows no silver-reducing property, and its cytoplasm appears packed with condensed silver-reducing substance. This cell is designated as type 3 (figs. 2 *B* and 4). Together with this type of cell are found similar cells having a more flattened, disklike shape, the content of which instead of being black appears brown, orange-brown or yellowish brown. This fading cell is designated as type 4 (figs. 3 *B* and 4). On comparing different varieties of this type of cell, one feels warranted in concluding that the fading and gradual disappearance of the silver-reducing property begin from the periphery of the cell and that the perinuclear zone is the last to lose its power to reduce silver. No evidence of excretion or of discharge of silver-reducing substance is found, and the cell itself does not perish in the course of this peculiar metamorphosis. It appears, then, that the transformation of the cytoplasmic silver-reducing substance into a nonreducing silver substance is executed by means of some intracellular chemical process. With the loss of silver–reducing substance, a cell is formed bearing only a faint trace of water blue, which may justly be called a chromophobe cell, or type 5 (figs. 3 *C* and 4). This cell has the form of an unevenly flattened disk with its inner convexity placed in close proximity to the basement membrane. Coexistent with the chromophobe cell, a cell is seen which is similar in appearance but shows more affinity for water blue. Its upper convexity appears more rounded, and it shows a rudimentary cytoplasmic protrusion directed toward the lumen of the intestine. This cell is designated as type 6. On tracing the next step in the metamorphosis of this cell, one finds that the cytoplasmic protrusion directed toward the lumen is longer and more pointed. This cell is designated as type 7. It gradually changes its entire orientation and fixes its anteroposterior axis perpendicularly to the basement membrane. It regains its full property of staining with water blue and finally shapes itself into an elongated cell which is indistinguishable from the indifferent epithelial cell and is generally regarded as the stem cell in the formation of new

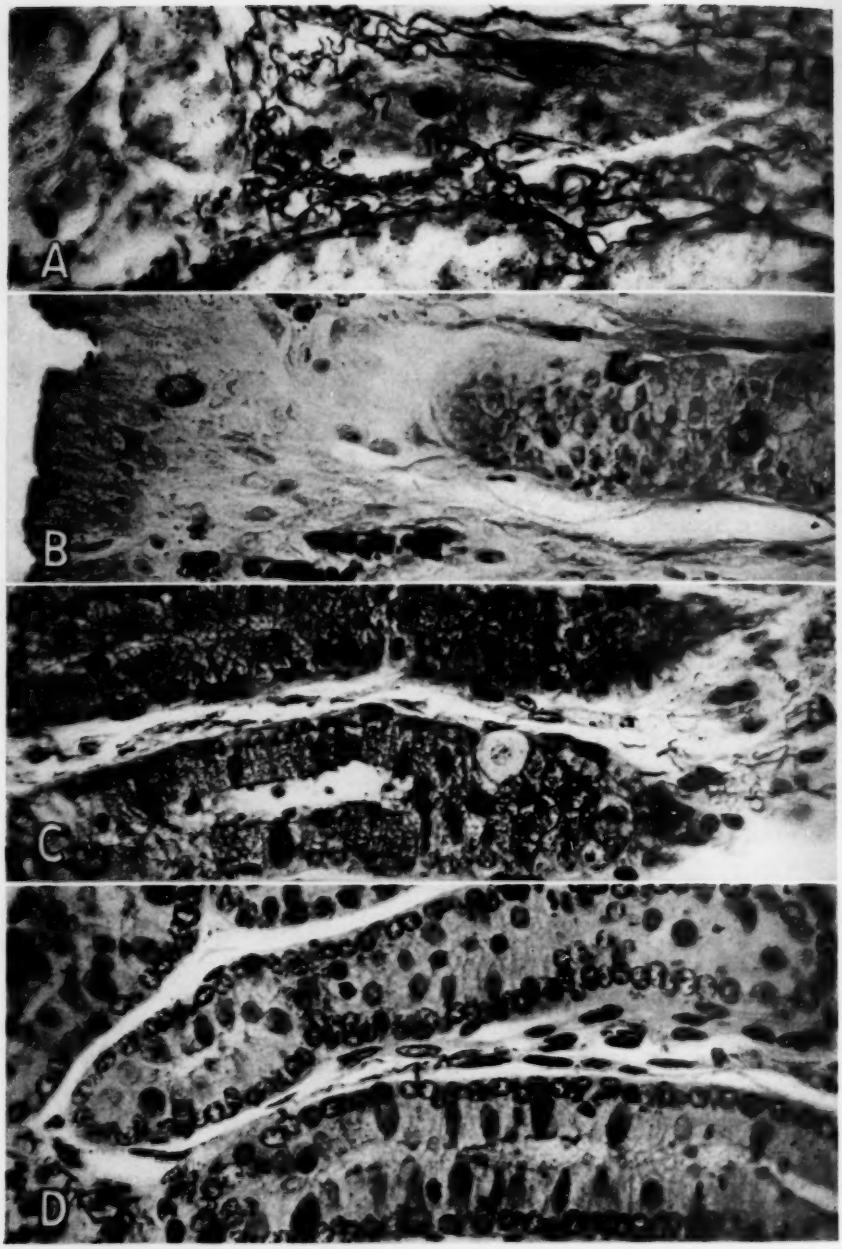

Fig. 3.—*A*, relation of the epithelial argentaffin cells to the basement membrane. *B*, further stage in transformation, with fading of argentaffinity. *C*, stage showing transformation of argentaffin cells into chromophobe cells. *D*, inverse relationship between mitoses and argentaffinity: mitoses numerous and argentaffin cells absent. (All figures from sigmoid; × 430.)

mucous cells. This cell is designated as type 8. The evidence afforded by further studies indicates that the indifferent cell formed as the result of the just described cytomorphosis is able to undergo differentiation and transform itself eventually into a fully developed mucous cell. Integration of the observations just reported (figs. 4 *II* and 12 *III*), which have been verified in a large variety of materials, permits the conclusion that they represent a chain of cellular changes which are fundamentally different from the cellular changes associated with the repeated successive phases of the secretory activity of the normal mucous cell.

The consensus is that the number of mitoses found in normal organoid tissue serves as an index of cellular efficiency in replacing worn out elements. It is believed that mitoses are not found in goblet mucous cells at all or are of very rare occurrence. Mitoses are observed in the indifferent cells of the sigmoid, but their number is certainly in disproportion to the wear and tear to which the mucosa of this part is subjected continuously. In an examination of thousands of serial sections not a single mitosis is found in true goblet cells. The number and distribution of the mitoses found in the indifferent epithelial cells differ in an unusual way. In following up a long series of sections one notes that many consecutive sections may be free from mitoses and that then mitoses appear in great numbers but are seen only in particular areas of the microscopic field. The unusual feature of these particular areas is that while they are rich in mitoses they show no argentaffin cells. Furthermore, when mitoses are looked for in areas rich in argentaffin cells, they are not found. It appears, therefore, that *the number of mitoses found in the indifferent cells is in inverse relation to the number of argentaffin cells found in the same area.* Figure 3 *D* serves to demonstrate the described relation between the number of mitoses in the indifferent cells and the number of typical argentaffin cells found in the same field. In this microscopic field there are more than 10 mitoses with not a single argentaffin cell present. Such a peculiar relationship indicates that when cytomorphosis of cell type 1-c proceeds normally there is no call for mitoses and that when cell type 1-c fails to continue its cytomorphosis mitoses are called on to compensate for the failure (fig. 4).

Silver reduction effects obtained with formerly used methods place in the foreground only typical argentaffin cells. The older methods (Fontana-Masson, Hasegawa and others) are worthless in demonstrating the genetic relations of argentaffin cells to other elements of the mucosa, and quantitative studies made with their aid fail to offer any reasonable explanation of the quantitative discrepancies observed. As a result of this the conclusion is drawn that the so-called argentaffin cell is a special cell, sui generis, mysterious in origin and fundamentally different in function. When the genetic relationship between mucous

and argentaffin cells is taken into account and when closely related cells, for instance, types 1-c, 2 and 3, are counted, the total count may be as high as 40 per cent of all cells. Such extensive cytomorphosis proceeding in the absence of mitotic activity in the same area is suggestive and certainly must be of definite biologic significance. It is worthy of note that when in a particular area cells of types 1-c, 2 and 3 are present, cells of types 4, 5 and 6 are very rare. The synchronous appearance of cells closely related only genetically signifies that cytomorphosis may affect certain areas only and at the same time.

As they stand, the observations reported leave no doubt that argentaffin cells are related genetically to mucous cells. Mucous cells are known to be sensitive to a number of chemical stimuli applied directly to the mucosa. It is conceivable, then, that other aspects of relationship between these two types of cells may be revealed by local application to the mucosa of chemical stimulants which are known to be specific in their action on mucous cells. In studies on the effect of stimuli locally applied to mucous cells Pavlov [6] and Pewsner [7] employed silver nitrate solution, Babkin [8] iodine dissolved in potassium iodide and Florey [9] mustard oil diluted with olive oil. The results of their studies show that application of these noxious substances is followed by a rapid flow of mucus, with evacuation of the mucous content by the cells. In the experiments about to be reported the effect of the following substances applied locally was studied: (1) mustard oil (3 per cent in olive oil), (2) 5 per cent silver nitrate, (3) aqueous solution of iodine, (4) 5 per cent lactic acid and (5) 20 per cent magnesium sulfate. All five of these substances produce an excessive flow of mucus and yet, in spite of the severity of the injury sustained, not all mucous cells lose their content. The cells which fail to empty themselves are similar in every respect to the cell type 1-c described. Their content is stained safranin–Van Dyke brown–orange, and their cytoplasm shows the presence of granular silver-reducing substance. It appears that, compared with cell type 1, these cells remain refractory and are not influenced even by the powerful stimulative action of the chemicals applied. These cells are apparently lacking in normal response to secretory stimuli. As to cells of types 2 and 3, or typical argentaffin cells, it is found that they cannot be forced to discharge or to expel their silver-reducing content. They retain their usual intensity of silver reduction and appear undisturbed by the drastic action of the chemicals employed.

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6. Pavlov, I. P.: Le travail des glandes digestives, translated by V. Pachon and J. Sabrazes, Paris, Masson & Cie, 1901.

7. Pewsner, M.: Berl. klin. Wchnschr. **44**:41, 1907.

8. Babkin, B. P., cited by Florey.[9]

9. Florey, H.: Brit. J. Exper. Path. **11**:348, 1930.

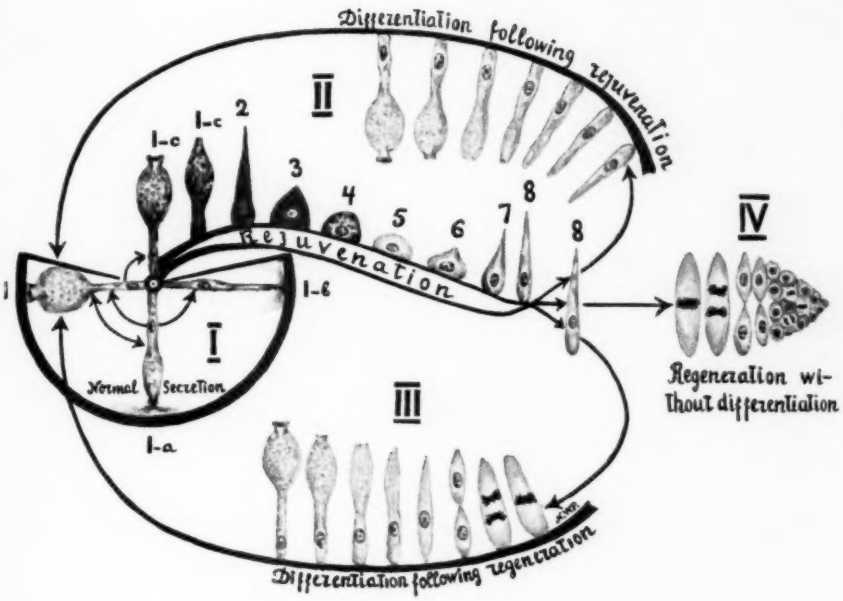

Fig. 4.—*I*, normal repeated successive secretory cycle of the goblet cell. *II*, rejuvenation cycle of a functionally exhausted goblet cell, followed by differentiation of the rejuvenated cell. *III*, rejuvenation cycle, followed by regeneration with consequent differentiation. *IV*, regeneration without consequent differentiation hyperplasia-neoplasia.

Cordier [10] reported that pilocarpine applied intravenously produces discharge of argentaffin granules and that elimination takes place at the apical pole, indicating the exocrine polarity of these cells. Though this observation is reported by an investigator whose contributions to the subject of argentaffin cells are most valuable, it requires further investigation. The question of the mechanism and of the specificity of the action of pilocarpine on the mucosa of the intestines is still unsettled. From the excellent experimental work of Florey [9] on the secretion of mucus by the colon it is clear that any influence that pilocarpine has on colonic secretion of mucus is totally different from that on glandular secretion, e. g., that of the salivary glands. To produce secretion of mucus in the colon, relatively enormous and repeated doses of pilocarpine must be injected intravenously and allowed to act over prolonged periods. Florey stated that pilocarpine applied locally to the rectum causes, after a few minutes, marked secretion of saliva, tears and bronchial mucus but an inappreciable secretion of intestinal mucus. It thus appears that claims of a specific action of pilocarpine on the intestinal mucosa lack a physiologic foundation. In the studies presented here pilocarpine was applied locally and intravenously, and in both instances argentaffin cells remaind unaltered. No discharge of silver-reducing substance was shown in apical or in basal parts of the cell, and this substance was never found discharged into the lumen of the sigmoid.

The state of argentaffin cells in different phases of digestion was studied, and the results fail to show any relation of these cells to digestion. Nothing strikingly different was found in experiments with complete starvation for seventy-two hours. It may be noted, however, that in the sigmoid of an animal starved for this period the number of argentaffin cells appears to be larger. The type of cell that appears prominent numerically is type 1-c. On comparing the effect of a diet of dry oats of short duration with that of a diet of carrots and lettuce it is observed that the former leads to an increase in the number of cells of type 1-c while the latter is accompanied by a decrease in the intensity of the silver reduction effect shown by cells of types 2, 3 and 4.

In not a single instance were epithelial argentaffin cells found moving or migrating through the basement membrane in a normal colon or in the colon of an experimental animal. When sections were cut parallel to the basement membrane and close to the basal parts of the gland, the argentaffin cells sometimes created the impression of being located behind the basement membrane, and that this was an artefact is apparent when the argyrophil reticulum is shown properly (fig. 3 *A*).

As part of the general plan the effect of delay in fixation was investigated.

10. Cordier, R.: Arch. de biol., Paris **36**:427, 1926.

A rabbit was kept fasting for twenty-four hours and then disposed of by air embolism. The abdomen was opened immediately, and a small segment of sigmoid was ligated on both sides, the ligation including the mesenteric blood supply. The segment was removed gently and without escape of blood from the removed segment or from the adjacent sigmoid left in. The abdomen was then closed and the animal left at room temperature. Four hours later the adjacent segment of the sigmoid was removed. In both instances the segments removed were fixed immediately in Helly's fluid and later on treated in exactly the same way. The difference in the microscopic pictures of the two segments removed revealed that the second segment failed to show in the tinctorial and impregnation results the degree of selectiveness manifested in the first specimen. Safranin staining effects remained unchanged in both segments, but the four hour delay in fixation had increased the number of cells which showed nonspecific silver reduction. It was not so much the cytoplasm as the nuclei of the cells which began to show this nonspecific reduction.

The findings indicate that metallic reduction tests are fully reliable only when they are applied to perfectly fresh tissues.

Summary.—Among the observations on the sigmoids of the 62 rabbits the following stand out as particularly important:

1. The mucous cells of the sigmoid are not identical in morphologic character and functional capacity. The nucleus and cytoplasm of a mucous cell of type 1-c shows the property of metallic reduction, and its mucous content is different tinctorially. Functionally, cells of this type are refractory and fail to respond to secretory stimuli.

2. Cells of type 1-c are not ordinary degenerating elements, and their elimination by necrobiosis or phagocytosis is not seen.

3. Argentaffin cells of types 2 and 3 are related genetically to cells of type 1-c.

4. Under normal circumstances the silver-reducing substance of argentaffin cells is never found excreted into the lumen of the intestine, and it is not demonstrated extracellularly at the base of the cell. This substance cannot be forced to leave the cell even under the influence of drastic irritants applied to the mucosa, and both local and intravenous applications of pilocarpine fail to affect these cells.

5. Argentaffin substance, probably a specific diphenol (Vialli and Erspamer[11]), is formed in the cell in the course of its cytomorphosis and is transformed into nonreducing substance by some intracellular chemical process (probably oxidation). As a result of this, the cell is transformed into a chromophobe element which in its shape and position is identical with the argentaffin cell.

6. In the course of further stages of cytomorphosis the cell returns to the state of an indifferent epithelial cell and is then indistinguishable from the rest of the undifferentiated elements of the epithelial lining.

11. Vialli, M., and Erspamer, V.: Ztschr. f. Zellforsch. u. mikr. Anat. **27**:81, 1937.

7. Under normal circumstances the number of argentaffin cells found varies; they may be numerous in some sections, and in other portions of the same block they may be absent entirely.

8. Their number is not influenced by local application of the chemicals tested and is not affected in a striking way by phases of digestion, starvation for seventy-two hours or types of diet employed in these studies.

9. The finding in one area of cells belonging to the same phase of cytomorphosis indicates that their cytomorphosis takes place at the same time and affects only particular areas.

10. The number of mitoses found in indifferent cells is in inverse relation to the number of argentaffin cells found in the same area. That the two processes are not observed at the same time is rather significant. It is apparent that when cytomorphosis fails regeneration by mitoses is called on to compensate this failure by production of new cells destined to reach the same goal of functional efficiency.

Chemically speaking, it is not proper to call cells which show reduction of silver and of other metallic salts argentaffin, argentochrome or metallaffin cells. Argentaffinity simply indicates that there are cells of entodermal, mesodermal and ectodermal origin which with specially adjusted methods give positive reduction of certain silver salts. Since with use of the same methods this reaction is obtained from a variety of genetically unrelated cells it offers little in itself to make understandable its significance in individual cases. It is only when it is studied in relation to the entire course of the cell's development or activity that its significance becomes understandable. In the studies presented attention was concentrated on the genetic relation of the argentaffin cell to other epithelial cells of the lining, and the results obtained show that in the sigmoid this cell simply represents a phase in the life cycle of the mucous cell.

With each point of observation substantiated by objective evidence, it is permissible to conclude that the normal secretory cycle of mucous cells is repeatedly manifested by successive phases in the activity of the same cell. When this cell finally reaches the stage of functional exhaustion, it does not perish. It becomes refractory, loses its response to secretory stimuli and undergoes a rearrangement or cytomorphosis which proceeds through various stages of dedifferentiation, with eventual return of the cell to normal secretory activity. The cytomorphosis signifies in reality functional rejuvenation of the once exhaustive mucous cell and reflects or discloses another mysterious faculty of living matter which may be called the phenomenon of functional rejuvenation.

In the light of this new concept the problem of the histogenesis and functional role of the so-called intestinal argentaffin cell finds a different

and more logical interpretation. It is apparent that the phenomenon of functional rejuvenation is of paramount importance in restoring the exhausted mucous cell to its normal function. Ability to rejuvenate makes unnecessary continuous replacement of worn-out elements by means of regeneration, and this makes understandable the discrepancies, so far unexplainable, between the scarcity of signs of cellular regeneration and the wear and tear to which the mucosa of the sigmoid is subjected continuously. This phenomenon is governed and controlled by its own mechanism and cannot be influenced by the experimental factors tried in these studies. As the appearance and disappearance of the substance possessing the property of reducing certain metallic salts is a manifestation of intracellular chemical processes which bear no relation to elaboration of a secretory product it is easily understood why this metallic salts–reducing substance is never found outside the cells and cannot be forced to leave the cells. This concept offers a reasonable explanation of the aforementioned observations on topographic distribution and quantitative interrelations between various types of cells found in the same field. When in a particular area cells fail to rejuvenate or the rejuvenated cells (type 8) fail to differentiate, regeneration takes place; this usually takes the normal course, but occasionally it takes a neoplastic course. The whole sequence of events and the results of deviations in the process of rejuvenation are shown in figure 4.

RESULTS FROM STUDY OF THE HUMAN LARGE INTESTINE

The material comprised fresh surgical tissues showing various inflammatory processes, benign polyps and carcinoma of different types. The results of these studies indicate that the human sigmoid shows the same two types of the cell's life cycle: one manifested by successive phases of secretory activity and the other by cytomorphosis of the nature of rejuvenation. In pathologic conditions these two cycles appear to be more complicated. The reaction on the part of the mesenchymal elements is the chief source of confusion. Degenerative processes come to the foreground, and a great number of cells of type 1-c fail to rejuvenate. Failing to regenerate, they degenerate. Their content escapes and is taken by phagocytes.

The benign tumors are represented in these studies by pedunculated polyps with the structure of well differentiated adenoma. This differentiation is manifested not only by the appearance of a glandular pattern but also by the demonstration of (*a*) the normal secretory cycle and (*b*) the rejuvenation cycle of the functionally exhausted mucous cell. There is no doubt that accumulation of mucous secretion in the closed interglandular spaces of polyps raises the pressure, causes trophic disturbances and is responsible for interference with the normal course of both cycles.

From comparative studies it is apparent that the capacity for growth in benign polyps is restricted not only by the extent of histologic perfection in glandular differentiation but even more by the ability of newly formed and fully developed mucous cells to rejuvenate normally. This conclusion appears to be warranted by the observation that new epithelial proliferation, as evidenced by regeneration through mitosis, is strikingly insignificant.

The different types of carcinoma are considered according to the classification of Ewing.[12] Adenoma destruens shows a variety of structures, ranging from completely preserved normal features of the glands to carcinoma growing in disorderly fashion, without any glandular differentiation. In each case of carcinoma control studies are made from segments of the intestine above and below the carcinomatous obstruction. Sections from adenomas show that in some parts of the growth both cycles proceed equally well. Fully developed mucous cells (type 1) contain normal-appearing, safranin-stainable mucus, and the cycle of rejuvenation is evidenced by the presence of argentaffin cells. A number of cells of type 1-c appear to be degenerating and, as free cells, are seen in the interglandular spaces. This is associated with local accumulation of phagocytes. In sections represented by disorderly growing cells, with no evidence of any glandular arrangement, nothing is found to indicate a manifestation of the two cycles shown by the mucous cell. Degeneration and mitotic activity here dominate the entire picture.

The particular feature of typical carcinoma is that no cells are shown containing safranin-stainable mucus or any signs of rejuvenation. Gelatinous carcinoma differs in many ways from the tumors discussed. In some areas it shows an alveolar structure consisting of poorly differentiated cells free from safranin-staining content. It is apparent that these cells possess the property of further differentiation, and, as a result of this, mucous cells containing safranin-stainable mucus are formed. These act as true secretory cells, profusely discharging their content into the free pericellular spaces. With no normal secretory stimuli on hand and lacking an outlet for the secretory product, each becomes gradually distended and ballooned and the nucleus is compressed into a signet ring form. Even under these abnormal circumstances the cells are capable of completing and repeating their cycle of secretion many times before they reach the stage of exhaustion. Instead of perishing, some of these exhausted cells undergo complete rejuvenation cytomorphosis, with transformation of the exhausted cells into typical argentaffin cells, as shown in figure 2 *C*. The significant feature of this photomicrograph is that it represents a section of infiltrating growth in the serous coat. With both cycles available there is no need for

12. Ewing, J.: Neoplastic Diseases: A Textbook on Tumors, Philadelphia, W. B. Saunders Company, 1934.

continuous regeneration, and it becomes understandable why mitotic activity is least prominent in gelatinous carcinoma. This explains also the tremendous capacity of the cells of gelatinous carcinoma to produce mucus, an excess of which is a specific feature of this type of carcinoma. These studies indicate that the index of malignancy cannot be evaluated on the basis of perfection in the degree of glandular differentiation. This index is reflected or better shown by the demonstration of an ability or an inability of the cells of a neoplasm to function (in the sense of successive cycles of secretion) and to rejuvenate (in the sense of rejuvenation of functionally exhausted cells). Demonstration of these two phenomena is impossible with the hematoxylin-eosin staining which is in use in present day methods of grading the malignancy of epithelial neoplasms.

As already mentioned, in each case of tumor growth a segment from above and one from below the obstruction were taken for examination. The comparison of these sections is very instructive. In many instances the segment from above the obstruction shows changes which are not observed in the segment below it. Above the obstruction cells of type 1-c are very numerous, while the argentaffin cells (types 3 and 4) are rare. This indicates that the functionally exhausted cells are unable to complete the normal cycle of rejuvenation under circumstances of continuous irritation and stasis. As a result of this the endogenous product of their gradual disintegration is taken up by macrophages, which are seen at first in the periglandular zone and later on in the deep part of the submucosa, and the content of such macrophages is browned or blackened by the silver nitrate methods employed. This explains why melanosis may be limited to a tiny area or to a patch, the neighboring mucosa being devoid of pigment. It is difficult to imagine that absorption from the intestine of some exogenous product could create such peculiarities in topographic distribution of melanosis. Below the obstruction rejuvenation proceeds uninterruptedly, and melanosis is not observed. In later life rejuvenation proceeds with greater difficulty, and it is not a coincidence that melanosis is observed most commonly in old age. Since the pigment found above the obstruction fails to give the prussian blue reaction it cannot be of hematogenous origin. These studies are in agreement with those by Stewart and Hickman,[13] but they offer a different explanation as to the cause of melanosis coli.

RESULTS FROM STUDY OF THE APPENDIX OF THE RABBIT

The appendix of a full grown young rabbit which had been kept fasting for twenty-four hours was considered as the normal control. An additional method employed which appeared to be of value is as

13. Stewart, M. J., and Hickman, E. M.: J. Path. & Bact. **34**:61, 1931.

follows: Sections are prepared in the usual way, treated with hydrazine hydrate–water blue reducer for ten minutes, rinsed quickly with distilled water, treated with a solution of gold chloride (1: 500) containing 0.5 per cent acetic acid for ten minutes, rinsed in water, dehydrated with alcohols and mounted in balsam. The studies included the influence of (1) fasting for seventy-two hours, (2) phases of digestion, (3) various diets and (4) local application of the substances employed in the sigmoid.

The results of studies of the sections show that (1) the type of epithelial lining is not the same in different areas; (2) the interrelation between the glandular layer and the underlying lymphoid tissue follows a definite pattern, and (3) the structure of the lymphoid tissue is different from that of the ordinary peripheral lymph node. The lymphoid tissue is represented by a continuous thick pad which separates the glandular layer from the muscular coat entirely. The surface of the lymphoid pad shows numerous conical papillary projections directed toward the lumen. In a quiescent and moderately relaxed organ these projections are 0.6 to 1.2 mm. high and 0.2 to 0.6 mm. wide at the base. Each conical formation projects into a domelike space, the roof of the dome being formed by the glandular layer of the mucosa (fig. 5 *A*). This topographic arrangement resembles somewhat the interrelation between the pyramid and the minor calyx of the kidney. The top of the roof is perforated with a glandular passage which contains a number of lateral glandular pouches. The glandular layer of the roof facing the free space of the dome shows numerous goblet cells and argentaffin cells but relatively few lymphocytes. The epithelial lining covering the conical lymphoid projections shows very rare mucous cells, equally rare argentaffin cells and very numerous lymphoid elements (figs. 5 *A* and *B* and 11 *C*). Whenever found, mucous cells show two cycles which are similar in every way to those observed in the mucous cells of the sigmoid. Similar results are also observed in animals under experimental conditions. Epithelial argentaffin cells are not found migrating through the basement membrane, and they cannot be forced to part with their argentaffin content under the influence of pilocarpine or drastic stimulants applied locally.

A difficult problem connected with studies of the appendix is offered by the structural peculiarities of the lymphoid tissue. This tissue shows both cortical and medullary substance, but the latter cannot be compared to the medullary substance of the peripheral lymph nodes. Mitoses are disseminated all over the lymphoid tissue, although they appear more numerous in the cortex. Both the cortex and the medullary substance are furnished with argyrophil reticulum, this being more prominent in the cortex. The medullary substance has its own vascular capillary plexus, which is not as rich as that in the cortex. The methods employed

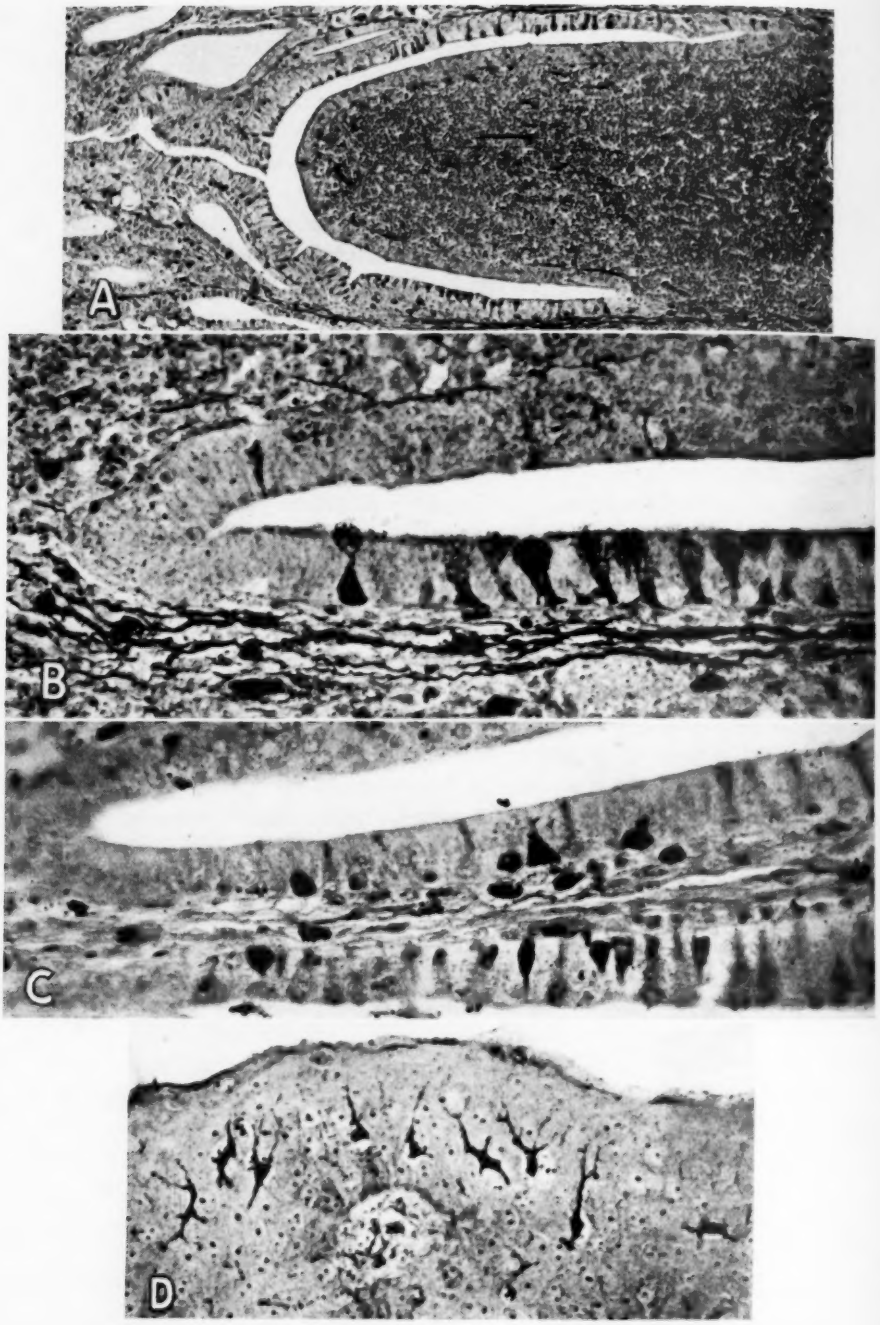

Fig. 5.—*A*, lymphoid projection in appendix showing difference in type of epithelial lining (× 100). *B*, first stage in transformation of goblet cells of appendix into argentaffin cells, showing difference in epithelial lining of Lieberkühn's gland and lymphoid projection. *C*, second stage in transformation. *D*, intraepidermal cells of Langerhans (× 430).

are helpful in differentiating various mesenchymal elements present in sections. Histiocytes are especially well shown. In certain phases of their activity these cells resemble closely the microglia cells which were demonstrated with the silver carbonate method by del Rio Hortega and Jimenez de Asua [14] in tumors, tubercles, hepatic lesions, the normal human kidney and in lymph follicles. The advantage of the methods used in the present work over the silver carbonate method is that they (especially method 3) reveal a definite range in tinctorial and impregnation differences which makes possible differentiation of various phases of the life and activity of the histiocyte (figs. 5 *C* and *D* and 11 *C*).

On treating sections with method 1 there are seen in the cortex and sometimes in the medullary substance peculiar looking cells, scattered singly or in groups. When seen in groups, some of them take water blue and some show silver reduction of a type differing from that shown by the argentaffin cells of Lieberkühn's glands. These cells are more numerous in the cortex (fig. 6 *B*), although quite often they are found in the medullary substance (fig. 6 *A*). When found in groups, they appear as giant cells with a voluminous crown of silver-reducing cells and small round cells taking water blue and containing a small confluent chromatin mass (fig. 6 *C*, right side). The peripheral cells appear to have an intimate connection with the pale cells scattered in the vicinity, which differ morphologically from the rest of the cellular elements. These formations are quite large, and sometimes one such formation is seen uninterruptedly in thirty consecutive sections 7 microns in thickness. At one level of sectioning cells are light blue, while at other levels they are light or dark brown. The content of the central cavity of the giant cell formation is either colorless or yellowish brown, or it consists of brown droplets or dark brown granules. In certain instances these formations show their own argyrophil reticulum. In tracing their structure in serial sections it is found that some of them have at one or another level of sectioning the unmistakable form of a tubule surrounded with fine reticulum (fig. 6 *C*, left side). In further sections of the same series the tubule gradually changes its shape; it becomes disfigured, and finally the whole structure acquires the appearance of multicellular formations arranged without any particular order. There can be no doubt that these formations are epithelial in nature and that they are endowed with some property of glandular differentiation. Topographic demonstration of these formations is best achieved with the gold chloride method described in the beginning of this section. With this method they stand up as black or dark brown cells on a light blue

14. del Rio Hortega, P., and Jimenez de Asua, E.: Arch. cardiol. y hemat. **2**:161, 1921. Jimenez de Asua, F.: Ztschr. f. d. ges. Neurol. u. Psychiat. **109**: 354, 1927.

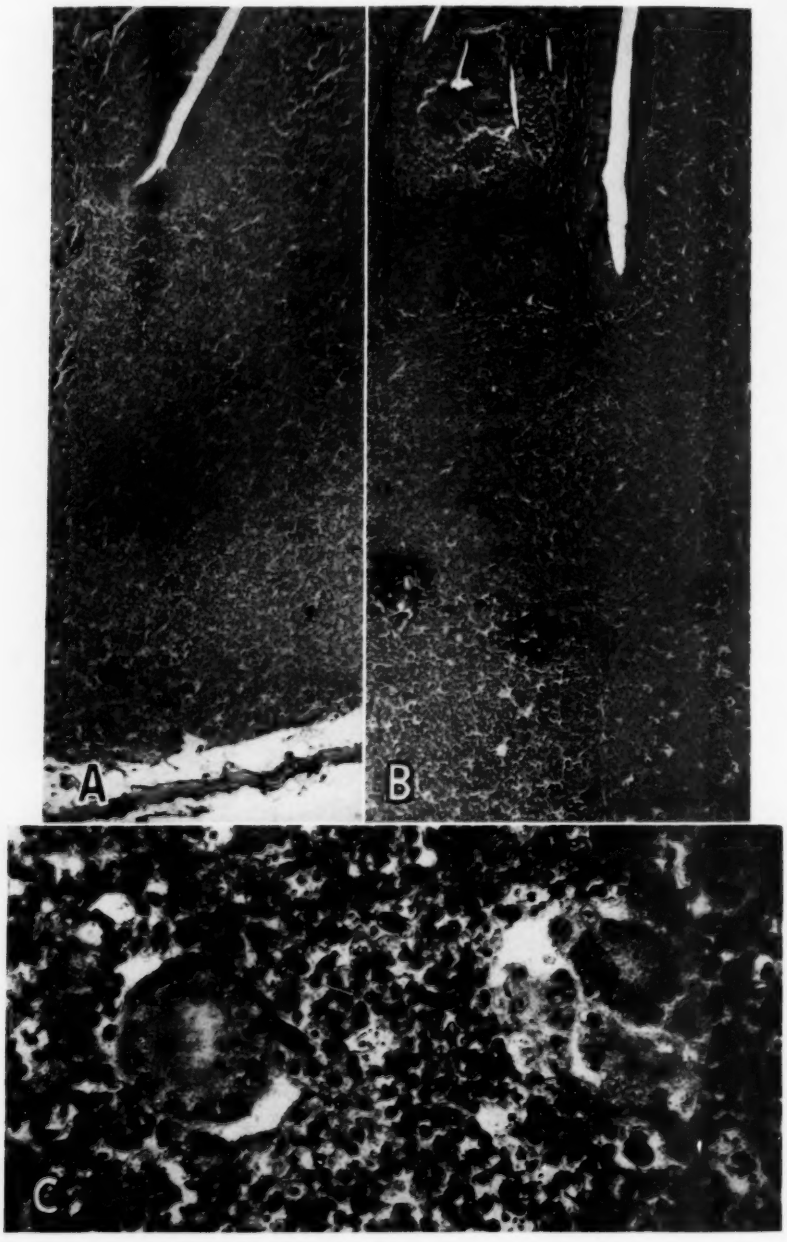

Fig. 6.—*A* and *B*, epithelial plaques in the cortical and medullary substance of the lymphoid tissue of the appendix (× 100). *C*, epithelial giant cell formations with tubular differentiation in the medullary substance of the lymphoid tissue of the appendix (× 430).

background (fig. 6 *A* and *B*). It is interesting that with this method the only content of some of these cells and the content of certain goblet cells of Lieberkühn's glands give gold reduction, while the mesenchymal cells show no signs of gold reduction. In some instances gold-reducing cells are not found; instead, there are seen finely granular cells of a signet ring appearance. In conclusion it may be said that in the rabbit the lymphoid tissue of the appendix is a lymphoepithelial tissue and resembles in every way the structure of the lymphoepithelial tissue of the bursa of Fabricius, which has been investigated and described with unsurpassed thoroughness by Jolly.[15] With method 3, the epithelial anlage is shown much more clearly than with the hematoxylin-eosin-azure stain used by me [16] in studies of the histogenesis of the thymus. With their location excluding external secretion, these cells with limited power of glandular differentiation pursue their own mysterious course of life. No cycle of rejuvenation similar to that observed in the goblet cells is disclosed. Only on rare occasions are cells found in the cortex which resemble the argentaffin cells of Lieberkühn's glands. In areas where the cellular and extracellular metallic salts–reducing content is abundant the phagocytes appear also in increased numbers, and the pigmentosis or melanosis of the lymphoid tissue in such instances is undoubtedly due to excessive accumulation and phagocytosis of a substance produced by the epithelial cells present in the lymphoid tissue. This is corroborated also by the negative results of the berlin blue reaction.

Summary.—From the observations in the appendixes of the 40 rabbits examined it is apparent that epithelial cells of the mucosa, which possess the property of reducing certain metallic salts, in their origin, sequence of intracellular changes and functional significance are identical with similar cells of the sigmoid. Differences in the morphologic appearance of argentaffin cells found in the appendix are closely related to differences in the appearance of mucous cells found in corresponding parts of the mucosa. For instance, in the epithelial coat of outer parts of the glands the mucous cells are low and small and here, too, only small argentaffin cells are found. The most convincing evidence of genetic interrelations between mucous and argentaffin cells is shown by the epithelial lining covering the conical lymphoid projections directed toward the lumen of the appendix. In this epithelial lining the goblet cells are very rare and here, again, the argentaffin cells are equally rare. As in the sigmoid, the pigmentosis or melanosis of the appendix is precipitated by interference with, or interruption of, the normal cycle of

15. Jolly, J.: Arch. d'anat. micr. **16**:362, 1914.

16. Popoff, N. W.: Proc. Soc. Exper. Biol. & Med. **24**:148, 1926; Arch. f. exper. Zellforsch. **4**:395, 1927.

rejuvenation of functionally exhausted mucous cells, and since the mucosa of the appendix is represented solely by one functional type of cell (mucous cell) it is understandable why the process of melanosis observed is most commonly found in the appendix. The finding of lymphoepithelial tissue in the appendix opens a new approach to studies of the physiologic and pathologic aspects of this organ. Figures 5 *B* and *C* and 11 *C* demonstrate nuclear and cytoplasmic changes associated with rejuvenation. Figures 5 *B* and 11 *C* correspond to an early stage and figure 5 *C* to a later stage of transformation of the functionally exhausted goblet cells into argentaffin cells.

RESULTS FROM STUDY OF THE HUMAN APPENDIX

The material examined included inflammatory conditions of acute, subacute and chronic types and a number of neoplastic processes (benign polyps and carcinoids). As stated previously, delay in fixation, even for four hours, interferes with the selectiveness of reduction methods, and for this reason only fresh surgical material, fixed immediately, was used in this work. Almost all appendixes removed surgically are pathologic in one way or another, and this excludes the possibility of making control studies on perfectly fresh normal organs removed from persons of different ages. In studies of lymphoid, and especially of lymphoepithelial, tissues the age of the animal, the state of nutrition and other factors concomitant with infection and toxemia are of great importance. Generally speaking, it is unreasonable to depend on clinical material in studies which pertain to fundamental problems of histophysiologic nature. With no way of knowing the nature of the inciting factor and the length of time it had been at work, it is difficult to tabulate qualitative and quantitative changes in an orderly manner. As far as general conclusions are concerned, it may be said that observed under pathologic conditions the essentials in the cycle of rejuvenation remain the same as when observed in normal mucosa. There is a great deal of individual variation, but the fundamental significance of the phenomenon of rejuvenation is unchanged. In cases in which appendical or fecal stasis is a predominant feature there is increase in the number of cells of type 1-c. With the safranin–silver–water blue method the mucous content of these cells stains differently, and their cytoplasm is filled with silver-reducing granules. It is apparent that because of stagnation and abnormalities in stimulation the mucous cells are unable to continue the normal cycle of secretion for their usual length of time. Functioning under such abnormal circumstances, they are unable to evacuate their content in normal fashion, and at the same time the exhausted cells, with unevacuated content, are not given proper conditions for completing the normal cycle of rejuvenation. This explains

the scarcity of argentaffin cells found and makes understandable why under such circumstances only cells of type 1-c and active phagocytes containing silver-reducing substance are prominent in the picture of appendical stasis.

As to the structure of lymphoid tissue, it may be said that pathologic material is unreliable for studies of this subject. In the majority of cases examined, such tissue is either absent (owing to complete atrophy), or it is distorted and altered to such an extent that little is left for dependable deduction. A few appendixes removed from children in cases of mistaken diagnosis of appendicitis were examined, and some of these showed well preserved lymphoid tissue. Studies of this material leave no doubt that human lymphoid tissue contains entodermal elements similar to those found in the appendix of the rabbit. The entodermal elements are observed most commonly in the cortex, while in the medullary substance they are discernible with great difficulty. The glandular differentiation observed in the lymphoepithelial tissue of the rabbit is not found in the human appendix. Attempts to demonstrate entodermal constituents of lymphoepithelial tissue with ordinary hematoxylin-eosin staining are futile, and in this work these elements are considered as entodermal only when corroborative evidence is offered by application of all four of the methods employed in studies of the lymphoepithelial tissue of the appendix of the rabbit.

A few words need to be said concerning the observations on neoplastic processes of the human appendix. A case of benign pedunculated polyp seated in the distal part of the organ (2 cm. from the tip) deserves particular attention. On comparing sections from the proximal and distal ends of the appendix with those from the polyp, one finds the rejuvenation cytomorphosis in all of the three sections examined. With an unobstructed outlet for mucous secretion in the proximal part of the appendix the lumens of the glands of Lieberkühn are free from stainable matter while the majority of the glandular cavities in the polyp are literally choked with mucus and brownish granular silver-reducing substance. Such areas appear to be invaded with a great number of active phagocytes. The epithelial lining is represented chiefly by cells of type 1-c, argentaffin cells being scarce. There are present, however, areas in the polyp which are indistinguishable from the mucosa of the appendix. As in the mucosa of the appendix, they show the usual goblet cells, argentaffin cells and cells of Paneth, and their glandular spaces are free from stagnant mucus and silver-reducing substance (fig. 11 *A*). In other words, the whole picture indicates that in such areas the process of rejuvenation pursues its course the same as in normal mucosa. Mitoses in polypous growths are very rare. It appears, then, that the life of such highly differentiated pedunculated growths is perpetuated solely by normal repetition of the secretory cycle followed by the uninterrupted cycle of rejuvenation.

There is a great deal of disagreement as to the origin of carcinoid tumors of the appendix. These tumors are specific in their topography and structure, and their cellular constituents show quite often the property of silver and chrome reduction. Masson[17] expressed the belief that migration of epithelial argentaffin cells occurs and that carcinoid tumors are formed as the result of budding at the tip of Lieberkühn's gland. A number of investigators failed, however, to demonstrate the phenomenon of budding described by Masson. The majority of carcinoids are removed at a time when little is left of the normal structure of the appendix: The lymphoid tissue is gone, the entire submucosa is replaced with tumor cells, and under such circumstances it is rather difficult to reconstruct a true picture of the changes which took place at the very beginning of the growth. In many publications it is stated that tumor cells are found immediately beneath the lining cells, and this is taken as evidence that the tumor has its source in the crypt of Lieberkühn. If a tumor growth originates in the Lieberkühn gland, it is strange that no one ever published a case of carcinoid having its seat in this gland and forming an everted growth. A great number of benign and malignant tumors definitely begin in glands of Lieberkühn. They grow as everted tumors, and though they may show the presence of argentaffin cells they are fundamentally different in behavior and structure from carcinoids. As a rule carcinoids grow as inverted tumors or, speaking more exactly, they grow beneath the glandular lining. The close proximity, however, of the protruding growth to the glandular lining cannot be considered as convincing evidence that the tumor originates from the lining of Lieberkühn's gland. Forbus[18] reported 6 cases of carcinoid, case 6 of his series being most interesting. The tumor was found in a patient having generalized miliary tuberculosis. It was located in the submucosa of the ileum, the mucosa being well preserved. Though no direct connection was found between it and the glands of the mucosa, the tumor cells extended close to the zone of the deepest crypts. No mention is made of the appearance of the lymphoid tissue in the region of the tumor or in Peyer's patches, the structure of which —as will be shown later—is not that of ordinary lymphoid tissue. In a majority of the reports of cases in the literature nothing is said about the lymphoid structure in the region of the tumors. Until the question of the initial relation of these tumors to the lymphoepithelial structures is given due consideration, nothing definite can be said as to the exact origin of carcinoids. In almost all the cases studied in the present work there was a failure to show any remnants of original lymphoid tissue, and attempts to find the source or seat of the primary growth seemed fruitless and futile. In only 1 of 6 cases examined were remnants found

17. Masson, P.: Am. J. Path. **4**:181, 1928.
18. Forbus, W. D.: Bull. Johns Hopkins Hosp. **37**:130, 1925.

which could be considered as original lymphoid tissue, and the intimate relation between the epithelial and the lymphoid tissue was found to be identical with that observed in the lymphoepithelial tissue of the appendix of the rabbit. It is worthy of note, too, that when a solid cord of carcinoid growth shows incipient phases of glandular differentiation these primitive glandular structures resemble in every way similar structures found in the lymphoepithelial tissue of the rabbit's appendix. In a case of carcinoid examined the epithelial structures showed complete glandular differentiation. Here the tubules were surrounded with argyrophil reticulum in the form of basement membrane, and the epithelial lining was furnished with terminal bars which were shown with unusual clarity by the silver methods employed in this work. No objective evidence was found to indicate that the argentaffinity shown quite often by carcinoid cells signifies degeneration, and it may be assumed that such argentaffinity is related to the phenomenon of rejuvenation demonstrated in epithelial cells of Lieberkühn's glands. Absence of mitotic actvity and the low grade of malignancy of these slow-growing tumors serve as an indirect argument in support of such a supposition. If this supposition is correct, then, applying a functional term, the carcinoid or argentaffin tumor should be called a rejuvenocytoma of the intestinal tract.

RESULTS OF STUDY OF THE SMALL INTESTINE OF THE RABBIT

The small intestine was the main object of my work on the terminal vascular system, and this offered an opportunity for studying argentaffin cells in a great variety of materials. In the series now being reported 50 rabbits were used. The presence of the valves of Kerkring and of villi contribute enormously to an increase of surface mucosa which physiologically and histologically differs from the mucosa of the colon. The epithelial lining is represented by the following types of cells: simple columnar cells with striated cuticular border, goblet and argentaffin cells, and Paneth cells. Lymphoid tissue is represented by solitary follicles scattered all over the intestine but more numerous in the distal part of the small intestine. Aggregated follicles, or patches of Peyer, occur as a rule in the ileum, and only very seldom are they seen in the rest of the small intestine.

With a genetic interrelationship between mucous and argentaffin cells in the colon and appendix firmly established, the entire problem of this research resolves itself into the question of whether a similar interrelationship exists in the small intestine. Here the number of mucous cells is much smaller than in the colon and appendix, and on comparing different parts of the small intestine one finds that they are more numerous in the distal part of the ileum and in the duodenal

papilla close to the ampulla Vateri. Placed between high columnar cells, the goblet mucous cells of the small intestine are smaller and taller than those in the colon and usually are scattered singly throughout the epithelial lining. As do those in the colon, they show two cycles: one manifested by successive repetitions of the process of normal secretion and the other by cytomorphosis associated with rejuvenation of refractory and functionally exhausted mucous cells and eventual return of the rejuvenated cells to normal secretion (fig. 12*II*). Since the mucous cells are less numerous and are scattered singly, the argentaffin cells are also found to be less numerous and scattered in the same way. In many instances cells of type 1-c retain the shape of goblet cells and stand out in distinct contrast to the surrounding columnar cells, which take only water blue. In the small intestine mucous cells are smaller and taller and argentaffin cells appear correspondingly more delicate and much more elongated than in the colon. The cytoplasmic continuations directed toward the lumen of the intestine are seen with unusual clarity, this variety of cells being much more predominant here than in the colon. In parts of the small intestine which are rich in mucous cells the argentaffin cells are observed in correspondingly greater numbers. This close quantitative interrelationship is particularly well shown in the duodenal papilla close to the ampulla Vateri. Judging by the number of argentaffin cells found in this highly important physiologic region, one decides that here rejuvenation compensates wear and tear with the utmost vigor. In not a single instance are argentaffin cells found migrating through the basement membrane. The argentaffin cells cannot be forced to part with their silver-reducing substance under the local effect of the following substances: epinephrine, physostigmine, histamine, pilocarpine, atropine, sodium nitrite, benzene benzoate, 75 per cent alcohol, mustard oil, aqueous solution of iodine, silver nitrate, palladium chloride, lactic acid or 20 per cent magnesium sulfate. The intravenous application of pilocarpine also fails to influence the content of argentaffin cells. The experiments demonstrating these facts were performed with the technic employed in the studies of the colon and appendix. The observations on the effects of starvation for seventy-two hours, of different types of diet and of various phases of digestion show no striking difference from the results obtained in the colon and appendix. In a great number of cases the small branch of the mesenteric artery supplying the segment under experimentation was visualized by an injection of india ink in a live animal, but no particular topographic relation of the argentaffin cells to the blood vessels was disclosed. The structure of Peyer's patches is that of lymphoepithelial tissue. Entodermal cells are found here, scattered singly or in multicellular formations, which are easily discernible with the four methods employed in

studies of lymphoepithelial tissue of the appendix. Multicellular epithelial plasmodial masses quite often show signs of glandular differentiation with formation of abortive tubules furnished with argyrophil reticulum. It is with great rarity that pigmentosis (melanosis) of the small intestine is found. Compared with those in the colon, mucous cells in the small intestine are much less numerous and here, too, lymphoepithelial tissue is found only in the distal part of the ileum (patches of Peyer) and is more insignificant in amount than in either the colon or the appendix. Scattered singly here and there, the mucous cells, even in cases of disturbance with rejuvenation, are unable to precipitate a local phagocytic reaction to the extent observed in the colon and appendix. Here, also, the motor activity of the villi and the absence of lateral pockets in Lieberkühn's glands prevent stagnation of products which are formed as the result of interruption of the rejuvenation cycle and which act as the inciting factor in mobilizing local phagocytic defense. These anatomic peculiarities shown by the small intestine are sufficient to explain why the pigmentosis or melanosis so commonly found in the colon and appendix is hardly ever observed in the small intestine.

CELLS OF PANETH

In a review of the literature no reference was found to positive impregnation of Paneth cells with silver methods. In an article on cells of Paneth, published in 1937, Hertzog [19] said that Paneth granules are not stained by silver salts. Mols [20] in studies on Paneth cells employed, in addition to other methods, the methods of Castro, Golgi and Cajal, but he made no reference to the effect of metallic salts on the granules of Paneth cells. No clearcut demonstration of secretory matter produced by cells of Paneth free in the cul-de-sac of a gland is found in the available literature. Mols [20] stated that he was able to see in the glandular cul-de-sac granules and filaments stainable by Mann's method. His main arguments, however, are more indirect, and his drawings are not clear enough. Maximow and Bloom [21] expressed the opinion that a discharge of granules into the lumen is rarely seen except under the influence of pilocarpine. Hertzog [19] stated that no granules were ever seen outside of the cell in the lumen of the crypt.

The results obtained with the methods used in the present work show (1) that the granules of Paneth cells are impregnable with silver (fig. 7 *A*) and (2) that the same silver-reducing product of secretion is demonstrable with utmost clarity in the lumens of the glands without any

19. Hertzog, A.: Am. J. Path. **13**:351, 1937.

20. Mols, G.: Arch. de biol., Paris **40**:111, 1930.

21. Maximow, A., and Bloom, W.: A Textbook of Histology, Philadelphia, W. B. Saunders Company, 1930.

application of pilocarpine. A photomicrograph (fig. 7 *B*) shows Paneth cells in a state of active secretion, and in both left and right lower corners are seen uninterrupted connections of secreting Paneth cells

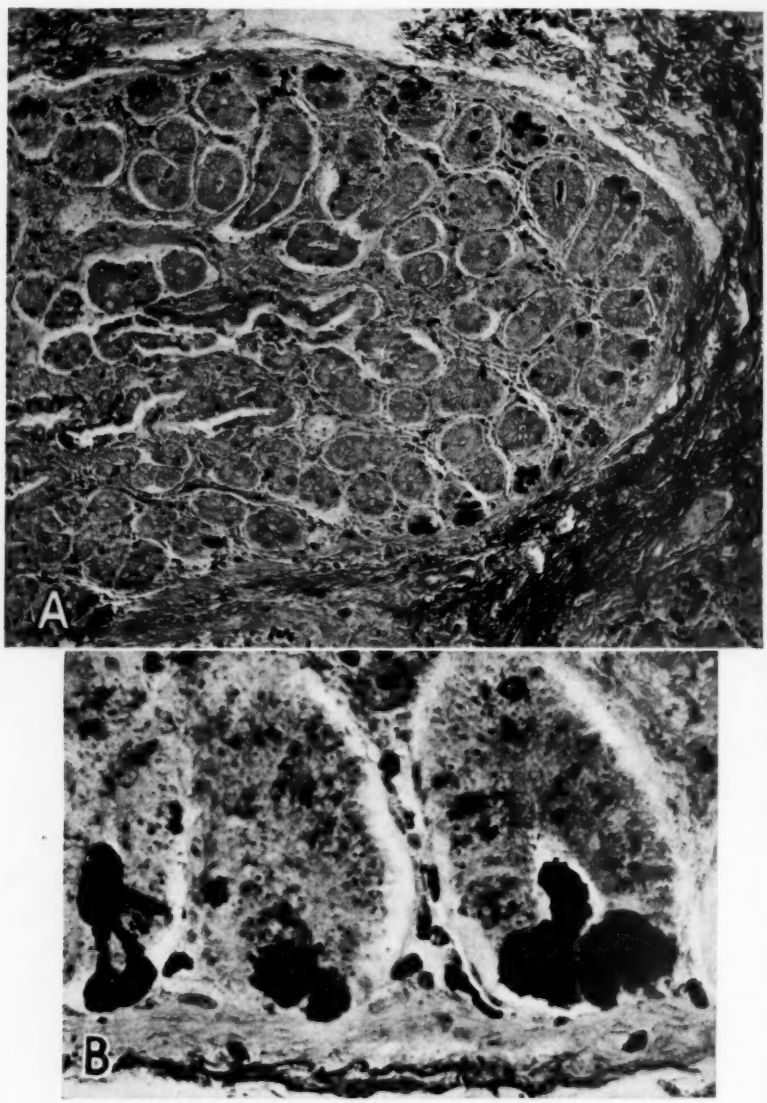

Fig. 7.—*A*, human jejunum with a crown of Paneth cells showing silver-reducing granules (× 100). *B*, human jejunum showing uninterrupted connections of Paneth cells, with their silver-reducing product filling the lumen of the gland. Note the appearance of the true argentaffin cell in the upper part of the gland on the right.

with their secretory product filling the lumen and giving a reaction of silver reduction similar to that shown by intracellular granules of Paneth cells. The observations in this work are significant for the following reasons: (1) They furnish a reliable method for demonstrating the intracellular and extracellular secretory product of Paneth cells, and (2) they offer additional evidence in support of a concept championed by Klein [22] and Bensley [23] to the effect that Paneth cells are zymogenic and have nothing to do with goblet cells. Bizzozero [24] claimed to have found transitional forms between Paneth cells and goblet cells. Prenant [25] regarded Paneth cells as mucous cells but as specific elements, different from the goblet cells, not as young goblet cells. Hertzog's conclusion is that the Paneth cell gives evidence of being mucoid in character rather than an independent zymogenic cell. One has to agree with Bensley that claims in regard to the specificity of the various methods employed in studies of Paneth cells are unjustifiable and that they have served only to add confusion to this subject. Furthermore, Bensley [23] stated that the difference in appearance of the granules found in Paneth cells is to be given a chemical rather than an architectural interpretation. From studies reported here it is obvious that the tinctorial and reduction effects obtained reflect in reality individual phases in the secretory cycle of the Paneth cell. With the water blue–silver method, depending on the phase of activity of the Paneth cells, granules may not be found at all, may take either water blue or silver or may show only clear vacuolar structures which do not take any stain. With the safranin–silver–water blue method the same results are observed, but the safranin never touches the content of Paneth cells, although it stains the mucous content of goblet cells most selectively. With the potassium bichromate–eosin–silver–water blue method, depending on the phase of activity, granules stain either blue, red or black. The cells of Paneth are secretory zymogenic cells, and they are entirely different from argentaffin cells. Their tinctorial and impregnation features reflect different repeated successive phases of secretion. The ordinary argentaffin cells are not secretory cells, and their tinctorial and impregnation features signify a specific process of rejuvenation of functionally exhausted mucous cells, a process which is not shown by the cells of Paneth.

RESULTS OF STUDY OF THE STOMACH OF THE RABBIT

Each stomach examined was opened immediately and washed with Helly's fluid. Trimmed portions were put in fresh Helly fixative for

22. Klein, S.: Am. J. Anat. **5**:315, 1906.
23. Bensley, R. R.: Anat. Rec. **2**:92, 1908.
24. Bizzozero, G.: Arch. f. mikr. Anat. **40**:325, 1892.
25. Prenant, A.: Compt. rend. Soc. de biol. **62**:1125, 1907.

twenty-four hours. Sections were taken from the cardiac portion (including the esophagus), from the fundus and from the pyloric region. The last part was trimmed in such a way that part of the stomach, the pylorus and the duodenal papilla were seen in the same section. The newly devised methods of staining and reduction gave constant, uniform and valuable results.

With the first, or silver–water blue, method, the parietal cells stand out clearly, resembling a bunch of grapes, each with a distinctly outlined (with silver) tubule-like projection directed from each cell toward the lumen and representing apparently the excretory part of the cell (figs. 10 *A* and 11 *D*). The effects of phases of digestion, of different diets and of starvation for twenty-four to seventy-two hours form the comparative material of the present studies. When the first method is applied to the stomach of an animal disposed of six hours after feeding the chief cells appear uniformly agranular and light blue, while the parietal cells vary in shade, some cells being greenish, some rusty yellowish green, some orange brown and some black.

With the third, or potassium bichromate–eosin–silver–water blue, method the differences in appearance of the parietal cells are much more conspicuous. It must be noted here that with hematoxylin-eosin staining the granules in the parietal cells are shown poorly and the individual differences in shades of eosin are too insignificant to be of value. With the third method, parietal cells appear either bluish green, bluish red, violet red, pinkish red or rusty orange brown. It is in cells of the last type that the first appearance of silver-reducing granules is observed (fig. 11 *D*). In the beginning the granules are small and are scattered all over the cell. The nucleus of such a cell loses its fine structural pattern, and its chromatin appears as a confluent mass taking water blue or eosin. In the course of further cytomorphosis the granules become coarser and more numerous, and finally the entire cell becomes loaded with silver-reducing substance to such an extent that no cytoplasmic structure is recognizable and the nucleus is seen only when the level of sectioning passes precisely through its middle. In this stage the argentaffin cell appears as a low conical cell with its broad base in close proximity to the basement membrane. When cut transversally, the cell appears as a angular cell (fig. 9) placed between zymogenic cells, and the only conclusion that could be drawn from observations on the process of its formation is that it is a parietal cell undergoing specific cytomorphosis. When the parietal cell reaches this stage of cytomorphosis, it begins to rotate and to retract toward the basement membrane (fig. 10 *A*). While retracting, it drags along its outer conical process, which now has the appearance of a tail (fig. 11 *D*). The cell shrinks and flattens and in the final step of retraction takes its position directly at the basement membrane. Its tail still recognizable, the cell is

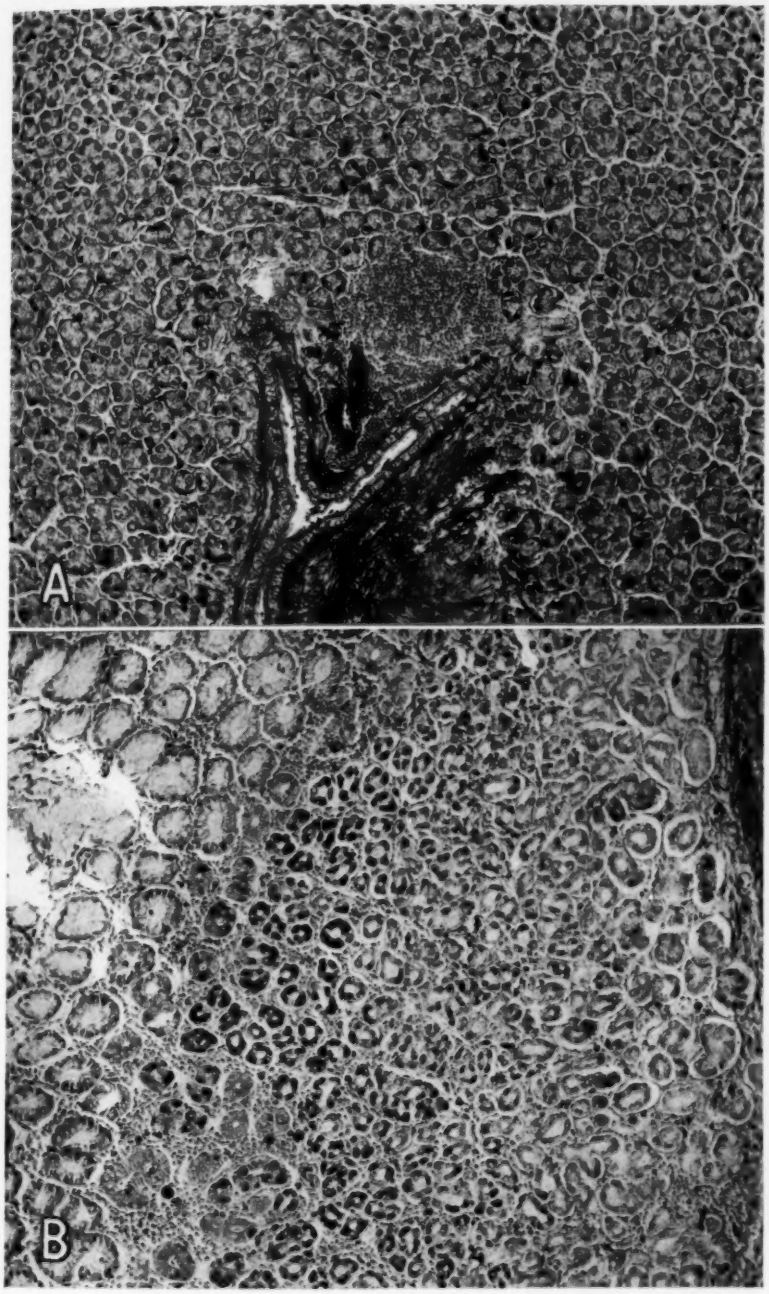

Fig. 8.—*A*, rabbit's stomach with evenly distributed argentaffin cells (× 100). *B*, human stomach with argentaffin cells limited to particular areas (× 100).

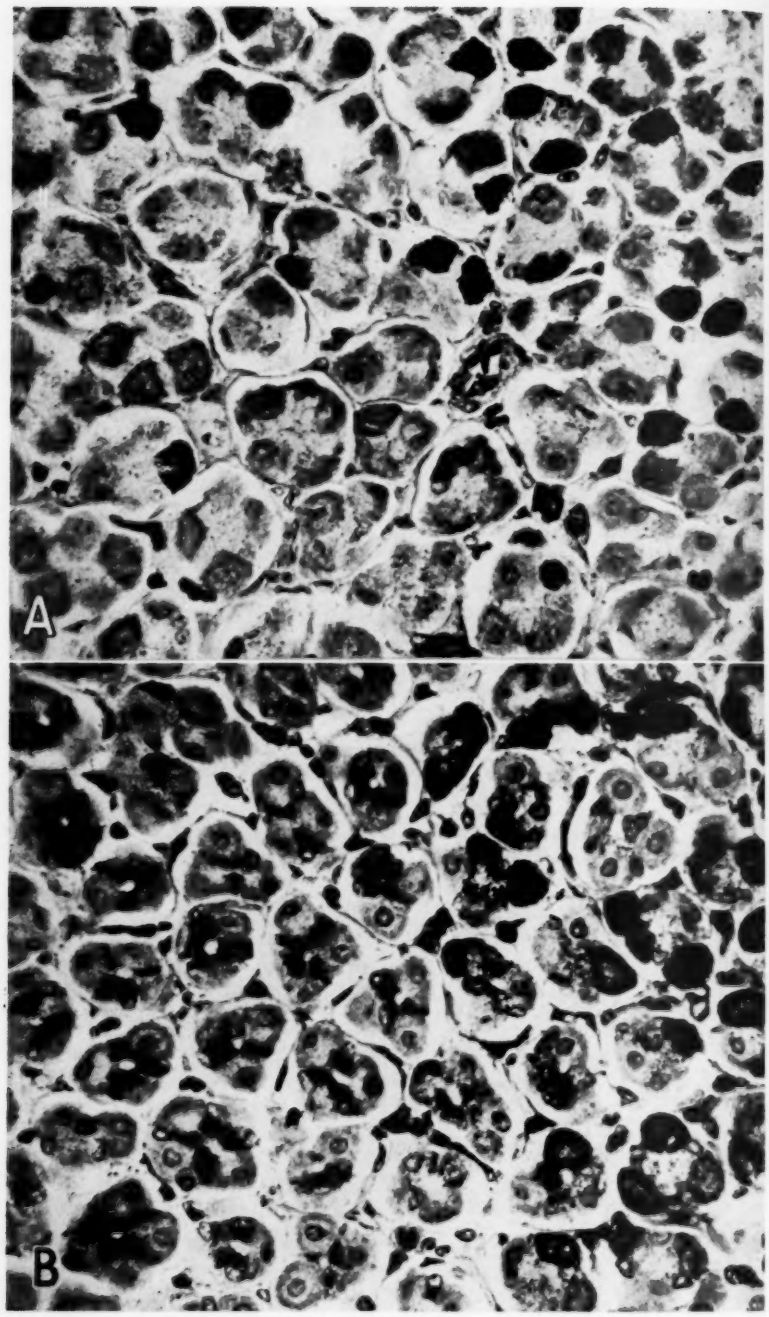

Fig. 9.—*A*, rabbit's stomach six hours after the rabbit was fed, showing early stage of transformation of parietal cells into argentaffin cells. The chief cells are empty (× 430). *B*, rabbit's stomach during starvation, showing an early stage of similar cytomorphosis. The chief cells are filled with granules (× 430).

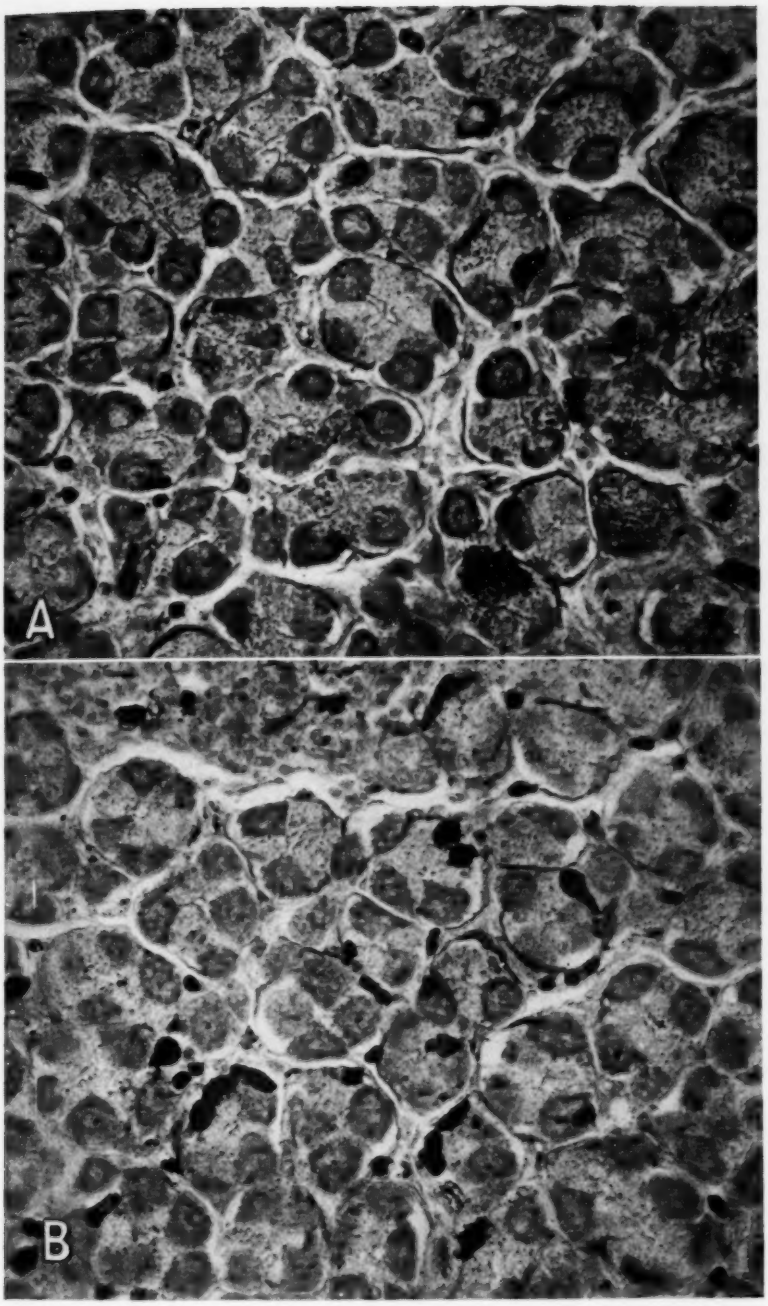

Fig. 10.—*A*, further stage in cytomorphosis of parietal cells. Note in the center a tubular projection connecting the parietal cells with the lumen of the gland (× 430). *B*, late stage in cytomorphosis, with argentaffin cells occupying a position directly at the basement membrane (× 430).

now comma-like in shape (fig. 10 *B*). On further shrinking it is gradually transformed into a small oval or round element (fig. 10 *B*), its argentaffin substance being much more abundant in the subnuclear region. In some of these cells the silver-reducing substance, instead of being jet black, becomes brown and rarefied. As a result of progressive fading, the cell is transformed finally into a chromophobe element which does not take water blue and contains no silver-reducing substance. This chromophobe cell gradually regains the property of staining with water blue, and in the course of further differentiation and reorientation it returns to the normal state of the parietal cell (fig. 12 *I*).

At no time is silver-reducing substance excreted by the argentaffin cell, nor is this substance found in the lumen of the gland or in any other pericellular zone. There are no signs that argentaffin cells have perished, and no evidence is ever found that they are eliminated by necrobiosis or phagocytosis. The histologic appearance of the chief cells and of the parietal cells varies in relation to the phase of digestion. These well known variations have nothing in common with the appearance and disappearance of silver-reducing substance observed in a certain type of parietal cell. The argentaffin substance is not influenced by phases of digestion, by starvation for periods of from twenty-four to seventy-two hours or by types of diet. The chief cells of the stomach never show signs of cytomorphosis similar to that observed in parietal cells. In figure 9 *A* (magnification, × 430—six hours after feeding) the chief cells are empty while in figure 9 *B* (magnification, × 430—starvation) they are heavily loaded with zymogenic granules which appear deep blue with a slight touch of silver. These photomicrographs represent two different phases of digestion and show that in spite of the difference in appearance of the chief cells the argentaffin cells are found in both photographs in the same number and are identical in appearance. It proves that neither their number nor their appearance is influenced by the stage of digestion and that the presence of argentaffin substance is not related to the production and secretion of digestive substance. The type, position and distribution of argentaffin cells are not the same everywhere: in one case (fig. 8 *A*—× 100) they are scattered all over and appear as small flattened or oval cells, closely adhering to the basement membrane; in another case (fig. 8 *B*—× 100) they are limited to certain areas and may appear as large triangular cells which differ from the parietal cells only by the presence of argentaffin substance. As in the intestine, mitoses are not found in the gland containing argentaffin cells but are numerous in the gland which shows no argentaffin cells. It is known that an occasional parietal cell of the stomach contains two nuclei, and it is not a coincidence that an occasional argentaffin cell with two nuclei

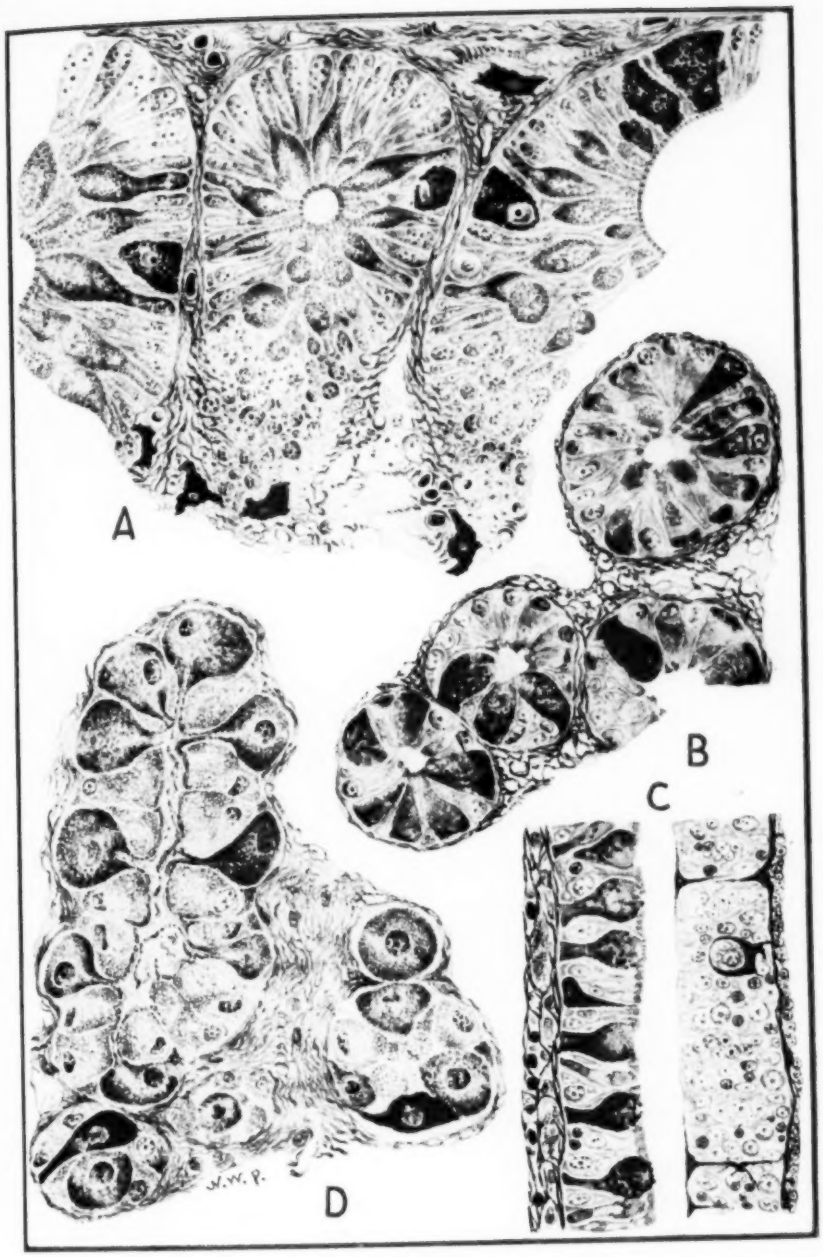

Fig. 11.—*A*, pedunculated polyp of a human appendix with argentaffin and Paneth cells (water blue-silver; × 450). *B*, rabbit's sigmoid showing early stage of transformation of mucous into argentaffin cells (safranin-water blue-silver; × 430). *C*, rabbit's appendix showing early stage of transformation of goblet into argentaffin cells. Note on the right side red-black histiocytes (eosin-water blue-silver; × 430). *D*, rabbit's stomach showing transformation of parietal into argentaffin cells (water blue-sliver; × 450).

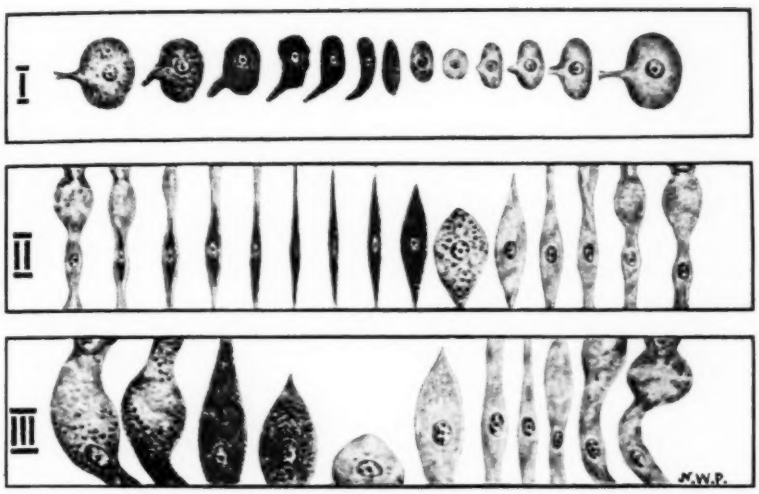

Fig. 12.—Rejuvenation cytomorphosis of (*I*) parietal cells of the stomach, (*II*) goblet cells of the small intestine and (*III*) goblet cells of the sigmoid.

is also found and only in the stomach. This is certainly a substantial argument in support of a genetic relationship between the parietal and the argentaffin cell of the stomach. Summarizing, then, the essential results of this work, one may conclude that the cytomorphosis observed in certain types of parietal cells is identical with that shown by the mucous cells of the intestine. It signifies rejuvenation of the functionally exhausted parietal cells and serves to explain a number of unsolved problems connected with the activity of parietal cells in normal and in pathologic conditions.

The space allotted does not permit discussion of other details, and only the following brief notes will be added. Brunner's glands take safranin and appear pinkish rose. Their secretory ducts contain both goblet and argentaffin cells, although neither of these types of cells is found in the gland itself. Pyloric and cardiac mucus-secreting glands show cytomorphosis of the rejuvenation type, this being especially pronounced in the stomach of the rat.

SUMMARY AND CONCLUSIONS

This work represents one more effort to shed light on hitherto unsolved problems concerning the origin and function of the argentochrome cells of the gastrointestinal tract, which were discovered in 1870 by Heidenhain [26] and later studied by Grutzner and Menzel,[27] Nussbaum,[28] Stohr [29] and others. In this work I have followed the traditions of my teacher, the late N. K. Kultschitzky,[30] who was an outstanding pioneer in this field of research. In approaching the problem careful attention was given to the work of all previous investigators. It is regrettable that discussion of their contributions is prevented by lack of space. It is satisfying, however, to know that there are in the literature reviews by Macklin and Macklin,[1] Schaffer,[31] Babkin,[32] Alvarez [33] and others of the histophysiology and pathology of the alimentary tract which are unsurpassed in their impartiality and completeness and which make unnecessary repetition here.

26. Heidenhain, R.: Arch. f. mikr. Anat. **6**:368, 1870.
27. Grutzner, M., and Menzel, H.: Arch. f. d. ges. Physiol. **20**:395, 1879.
28. Nussbaum, M.: Arch. f. mikr. Anat. **16**:532, 1879.
29. Stohr, P.: Arch. f. mikr. Anat. **20**:221, 1882.
30. Kultschitzky, N. K.: Arch. f. mikr. Anat. **49**:7, 1897.
31. Schaffer, J.: Das Epithelgewebe, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 2.
32. Babkin, B. P.: Die äussere Sekretion der Verdauungsdrüsen, in Gildemeister, M., and others: Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere, ed. 2, Berlin, Julius Springer, 1928, vol. 15.
33. Alvarez, W. C.: The Mechanics of the Digestive Tract, ed. 2, New York, Paul B. Hoeber, Inc., 1928.

From the studies presented in this paper it appears that argentochrome cells are not exocrine, endocrine or neurocrine. The objective findings in studies of 92 rabbits under normal and experimental conditions and of a considerable variety of human tissues are summarized in the following conclusions:

1. New, simple, rapid methods of staining and reduction have been developed which have proved of particular value in demonstrating a genetic relationship between the mucous and argentochrome cells of the intestine and between the parietal and argentochrome cells of the stomach. The methods formerly employed in the studies of argentaffin cells are cumbersome and time consuming and are worthless in demonstrating this genetic relationship.

2. Under normal circumstances the mucous cell of the intestine passes through successive phases of secretory activity repeatedly, and when finally the cell reaches the stage of functional exhaustion it does not perish. It becomes refractory, loses its response to normal and to artificially applied secretory stimuli and undergoes a rearrangement or cytomorphosis, manifested by the appearance, accumulation and gradual disappearance of substances which have the property of reducing metallic salts. In the course of this cytomorphosis the cell changes its position, dedifferentiates and returns eventually to the state of a normal secreting cell. This cytomorphosis of the functionally exhausted mucous cell is designated as a phenomenon of functional rejuvenation, and the entodermal argentochrome cell may be called a rejuvenocyte.

3. The rejuvenation is accompanied by certain cytoplasmic and nuclear changes, and the cytomorphosis observed in the course of rejuvenation is a manifestation of specific intracellular chemical processes and bears no relation to the elaboration or secretion of any product in the sense of exocrine or endocrine secretion.

4. The existence of such cytomorphosis is supported by the following findings:

(*a*) With the methods employed, definite intermediary forms are found between the functionally exhausted mucous cells, argentaffin cells and chromophobe cells.

(*b*) The silver-reducing substance is never excreted into the lumen of the intestine and is not found extracellularly.

(*c*) These cells cannot be forced to evacuate this substance by application to the mucosa of drastic irritants and other chemicals, and pilocarpine, applied locally or intravenously, fails to affect these cells.

(*d*) The number of argentaffin cells is not influenced by phases of digestion, by types of diet or by nineteen different chemical substances that were applied.

(*e*) The number of mitoses is in inverse relation to the number of argentaffin cells found in the same area, indicating that whenever rejuvenation fails regeneration by mitosis is called on to compensate this failure by the production of new cells destined to reach the same goal of functional efficiency.

(*f*) The epithelial argentaffin cells are never found migrating through the basement membrane, and extraglandular argentaffin cells are either mesenchymal or ectodermal.

(*g*) When found extraglandularly in the appendix or ileum, some argentaffin cells may be of epithelial nature, for the lymphoid tissue of the appendix and of Peyer's patches is in reality lymphoepithelial tissue, which resembles closely the lymphoepithelial tissue of the bursa of Fabricius. This offers a new approach to studies on the origin of extraglandular carcinoid tumors.

5. Argentaffin cells are found in benign pedunculated polyps and in some portions of malignant tumors of the intestine. In their origin and significance they are similar to argentaffin cells in the normal intestine, and it may be said that whenever there are in tumors mucous cells capable of function and rejuvenation there are found argentaffin cells also. The characteristic feature of benign polyps is that the rejuvenation cycle proceeds normally and mitoses are rare. When, owing to any cause, rejuvenation fails, the cells begin to regenerate, and the process of exuberant regeneration may take the course of a malignant neoplasm. It is not the degree of glandular differentiation that retards the malignancy of a growth. It is the ability of the cells to function and to rejuvenate that makes a tumor orderly and slow growing.

6. When mucous cells are called on to perform their function and to rejuvenate under unfavorable circumstances, such as advanced age, continuous irritation and stasis—for instance, in the colonic segment above a carcinomatous obstruction—the cycle of rejuvenation of the functionally exhausted cells is interfered with or interrupted. As a result of this, the endogenous product of their gradual disintegration is taken up by macrophages, which are seen at first only around the affected gland but later on in the corresponding deep part of the submucosa. This leads to patchy pigmentosis or melanosis, which is not found below the obstruction, where rejuvenation is not interfered with and consequently proceeds in the usual way. This serves to explain the origin and topographic peculiarities in the distribution of intestinal melanosis. The mucosa of the colon and appendix consists chiefly of mucous cells, while the mucosa of the small intestine consists of different epithelial cells and shows a much smaller number of mucous cells scattered here and there. This makes understandable why melanosis above the ileocecal valve is hardly ever observed.

7. The methods employed show the hitherto undemonstrable capacity of Paneth cells to reduce metallic salts. The results obtained indicate that in certain phases of secretory activity the granules of Paneth cells are impregnable with silver nitrate and that the same silver-reducing product of secretion is demonstrable with utmost clarity in the lumen of the gland. The cells of Paneth are secretory zymogenic cells, and they do not show the cycle of rejuvenation observed in mucous cells. They have nothing in common with mucous and argentaffin cells.

8. The methods employed demonstrate a genetic interrelationship between parietal cells of a certain type, argentaffin cells and chromophobe cells of the stomach. They show that the functionally exhausted parietal cell, instead of perishing, undergoes cytomorphosis, and the argentaffinity observed signifies the phenomenon of functional rejuvenation. As in the intestine, the argentaffin substance is not excreted by the cell and is never found outside the cell. The number of argentaffin cells is not influenced by phases of digestion, and argentaffinity is not related to the production and excretion of exocrine or endocrine substance. When passing through the stage of rejuvenation the parietal cell is relieved of its specific functional duty. With ordinary methods, the parietal cell in the early stages of rejuvenation does not reveal peculiar features; in other words, these older methods are unable to reflect the state and behavior of parietal cells in various physiologic and pathologic conditions.

9. Both pyloric and cardiac mucus-secreting glands show argentaffin cells of the rejuvenation type.

10. Excretory ducts of Brunner's glands are furnished with the goblet cells, and they show the presence of argentaffin cells.

11. In control studies the new methods were applied to all tissues of the animal body, and the results obtained are very interesting. Method 3 is especially valuable when applied to the hypophysis, in which it shows four distinct type of cells: chromophobe, light blue, red and argentaffin. The significance of such tinctorial and reduction effects is a problem in itself. These methods also permit differentiation of mesenchymal elements in relation to the phase of their life and activity, and they are valuable for studies of the intraepidermal cells of Langerhans which with these methods behave like regular histiocytes.

In conclusion: There are cells of merocrine secretion (the mucous cells of the gastrointestinal tract and the parietal cells of the stomach) which have the biologic faculty of maintaining their longevity and usefulness by cyclic returns, when functionally exhausted, to their primitive afunctional state, each return being followed by progressive differentiation and full recovery of their original efficiency. Argentaffinity, or metallaffinity, demonstrable with histochemical methods, reflects simply a

stage in the rejuvenation of certain functionally exhausted and refractory entodermal cells and bears no relation to exocrine, endocrine or neurocrine secretion. The significance of the observations reported with the new methods is discussed with reference to the following problems: (1) the general life cycle of certain highly differentiated cells; (2) the functional endurance of such cells in the absence of any signs of regeneration; (3) inability of some fully developed cells which have approached the rejuvenation stage to react to normal and artificially applied stimuli; (4) normal compensatory regeneration and focal neoplastic proliferation caused by any intrinsic or extrinsic factor interfering with the rejuvenation cycle or causing a cessation of it; (5) melanosis coli, precipitated by local disturbances, with rejuvenation of the goblet cells; (6) the lymphoepithelial nature of the lymphoid tissue of the appendix and of Peyer's patches and (7) additional evidence in favor of the zymogenic nature of the cells of Paneth.

EFFECT OF THYROID FEEDING ON THE REMOVAL OF CHOLESTEROL

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It is known from the work of Murata and Kataoka,[1] Liebig,[2] Turner,[3] Page and Bernhard [4] and Menne, Beeman and Labby [5] that administration of desiccated thyroid and thyroid-stimulating iodine compounds may prevent experimental atherosclerosis in rabbits. It is possible that, in addition to the lowering of the level of the blood cholesterol described by many authors, there is a direct stimulation of the macrophages in the aortic wall which, as has been shown by Anitschkow [6] and Leary,[7] take up deposited cholesterol and tend to dispose of it. In either case, whether the mechanism of thyroid protection from atheroma is a lowering of the level of blood cholesterol or some local effect, interest attaches to the effect of thyroid on the ability of cells to dispose of or destroy cholesterol; for any reduction in blood cholesterol must be caused by some cellular process in the liver or in the reticuloendothelial system. That the reticuloendothelial and macrophage system is intimately concerned in the handling of cholesterol in the body is indicated by the work of Anitschkow, Chalatow,[8] Kimmelstiel and Laas,[9] Thannhauser and Magendanz [10] and others.

In order to reveal the action of thyroid on cells phagocytosing cholesterol, a study was made of the effect of thyroid feeding on the removal of experimental intracutaneous cholesterol deposits.

METHODS AND TECHNIC

The cholesterol was injected in two different ways, suspended in water and suspended in olive oil. The water suspension was made by dissolving 0.5 Gm. of cholesterol cystals in 10 cc. of acetone. This was added to 50 cc. of a 0.5 solution of gelatin in water. The acetone was removed and the suspension concen-

From the United States Marine Hospital.

1. Murata, M., and Kataoka, S.: Verhandl. d. jap. path. Gesellsch. **7**:27, 1927.
2. Liebig, H.: Arch. f. exper. Path. u. Pharmakol. **195**:265, 1930.
3. Turner, K.: J. Exper. Med. **58**:115, 1933; **62**:721, 1935.
4. Page, I., and Bernhard, W. G.: Arch. Path. **19**:530, 1935.
5. Menne, A.; Beeman, J., and Labby, D.: Arch. Path. **24**:612, 1937.
6. Anitschkow, N.: Verhandl. d. deutsch. path. Gesellsch. **23**:473, 1928.
7. Leary, T.: Arch. Path. **21**:419 and 459, 1936.
8. Chalatow, S.: Beitr. z. path. Anat. u. z. allg. Path. **47**:85, 1914.
9. Kimmelstiel, P., and Laas, E.: Beitr. z. path. Anat. u. z. allg. Path. **93**: 147, 1934.
10. Thannhauser, S., and Magendanz, A.: Ann. Int. Med. **11**:1662, 1938.

trated by boiling. After the suspension had been filtered through dense paper the concentration of cholesterol was 12 mg. per cubic centimeter. Such a suspension may be injected through a 26 gage needle. The oil solution contained 0.5 Gm. of cholesterol in 12 cc. of olive oil. This was heated till clear and then was chilled and shaken until very fine crystals formed. It was injected at room temperature.

In preliminary experiments it was found that more than 0.6 cc. of the water suspension and more than 0.2 cc. of the oil suspension when injected intradermally would produce a nodule the contents of which would slough out in seven to eight days. Sloughing out of the nodule occurred also if the injection was given too superficially. If the cholesterol was injected beneath the dermis, it was spread by the movement of the skin to such an extent that no definite nodule formed.

Four adult rabbits were given intradermal injections of 0.2 cc. and 0.4 cc. of the water suspension in the clipped flank. There was a control injection of 0.5 cc. of the gelatin. The cholesterol produced firm lentil-shaped nodules, 0.5 and 0.6 cm. in diameter, while the control injection vanished within twenty-four hours. Tablets of commercial iodothyroglobulin were ground into a suspension and administered by means of a soft rubber catheter daily to 2 of the 4 rabbits. The rabbits received iodothyroglobulin equivalent to 3 to 5 grains (0.2 to 0.3 Gm.) of desiccated thyroid daily. This dosage produced loss of weight and emaciation. Such doses have been shown by Turner [3] and by Menne, Beeman and Labby [5] to be sufficient to protect against atheroma.

The second group was composed of 10 healthy rabbits which had been discarded after previous bleedings and experiments. Each of these animals was given five injections intradermally, making a row parallel to the spine. Fifteen days later, another series of five lesions was placed on the opposite side in every animal in this group except 3. Each injection consisted of 0.15 cc. of the olive oil suspension. Control injections of 0.15 cc. of olive oil were given. Test rabbits received 4 to 6 grains (0.25 to 0.4 Gm.) of desiccated thyroid daily. When the drop in weight became too rapid, the administration of thyroid was discontinued for several days. There were 5 test rabbits and 5 controls. Two test animals died on the eleventh day from overdosage of thyroid. Lesions were removed after they had been present for fifteen days and after thirty days. Lesions from 1 test animal and 2 controls were removed at twenty-eight days and fifty-eight days, respectively.

RESULTS

The 4 rabbits given the watery suspension showed but little inflammatory change about the points of injection. The 0.2 cc. nodules were removed from all 4 rabbits after fifteen days. At this time the nodules had decreased from about 5 mm. to about 3 mm. in diameter. There was no difference in the size of nodules on controls and thyroid-fed animals. One test animal died two days later, and the 0.4 cc. lesion was removed. The remaining 3 rabbits were followed for two weeks more, until the lesions were barely palpable. The lesions on the 3 animals were almost identical in size at this time, again showing no effect of the thyroid feeding.

After injection of the oil suspension there occurred an inflammatory area 1 cm. in diameter, which in the course of several days contracted to a small nodule, 5 to 6 mm. in diameter. The impression was gained that in the thyroid-fed rabbits the olive oil control and the first inflam-

EXPLANATION OF FIGURE

A, lesion eleven days old from a test rabbit (oil suspension); hematoxylin and eosin; × 175. The photomicrograph shows the cholesterol spaces with protein between them but with no cellular reaction. The darker areas are collagen fibers, which take a deep hematoxylin stain.

B, lesion two weeks old from a control rabbit (oil suspension); hematoxylin and eosin; × 540. The histiocytes are shown collecting about and between the cholesterol crystals and forming giant cells.

C, lesion fifteen days old from a test rabbit (water suspension); frozen section stained with sudan III and photographed between partially crossed polarizers; × 120. It shows the distribution of sudan-stained macrophages about the central crystal deposit. It may be noted that although the macrophages stain deeply with sudan III there is little anisotropic material within them.

D, lesion thirty days old from a test rabbit (water suspension); hematoxylin and eosin; × 540. The very large giant cell with eosinophilic peripheral cytoplasm and the finely granular cytoplasmic zone is shown together with lipoid-laden macrophages and smaller giant cells.


A
B
C
D

matory phase of the lesions disappeared more rapidly than in the controls. After about a week the lesions on control and test animals became the same size and remained so. There was some variation in size between lesions of the same duration on the same animal. One control rabbit of this group had unusually large lesions, which remained larger than those of the other controls.

With the exception noted, it was clear that there was no significant difference in the size and character of the lesions on control and test animals.

MICROSCOPIC OBSERVATIONS

The microscopic observations are based on sections from: (*a*) fifteen day lesions of 2 control and 2 test animals; (*b*) eleven day lesions from 2 test animals; (*c*) fifteen day lesions from 2 test animals and 3 controls; (*d*) thirty day lesions from 2 test animals and 3 controls; (*e*) twenty-eight and fifty-eight day lesions from 1 test animal and 2 controls. Each lesion was studied in both frozen and paraffin sections. The frozen sections were examined with sudan IV and between polarizers.

During the first week the lesions showed little reaction about the deposits of crystals. In several lesions there were numerous polymorphonuclear leukocytes, but their presence was interpreted as a reaction to accidental infection. *A* in the figure shows the absence of cellular reaction after eleven days. There is only a slight suggestion of the marked necrosis described in such lesions by Basten.[11]

After the first week there was mobilization of histiocytes. These cells, which are shown in *B* in the figure, applied themselves to the surfaces of the cholesterol, sent out processes which enveloped the crystals and in other places fused to form giant cells. In the lesions produced by the water suspension many of these early cells degenerated, forming a cellular débris, which was removed by later cells. In the lesions produced by the oil suspension (*B* in the figure) this degeneration was absent. Aside from this difference the oil suspension lesions ran parallel with the water suspension lesions. The cytoplasm of the histiocytes and of the giant cells they formed was at first eosinophilic with a very homogeneous appearance. In the lesions from two to three weeks old, "foamy" areas with neutral staining properties appeared within individual cells and within the giant cells. The giant cell in *D* in the figure shows this foamy change in the central region occupied by the nuclei. In almost every case the nuclei of the giant cells were within this area and usually distributed along its periphery. This tendency may be clearly seen in *D*. The foamy areas of the giant cells under the highest resolving powers of the microscope were identical with the contents of the so-called "foam cells." In the oldest lesions

11. Basten, G.: Virchows Arch. f. path. Anat. **220**:776, 1915.

there were few giant cells, the predominating cell being a rounded, well filled macrophage with foamy or granular, neutral cytoplasm.

Frozen sections of the lesions with an early macrophage response showed numerous intracellular sudanophilic droplets, which were for the most part optically inactive. This is shown in *C* in the figure. This lesion was taken from a rabbit which received the watery suspension of cholesterol. There is, therefore, no possibility that these droplets were phagocytosed olive oil. It must follow, then, that one of the earliest steps in the removal of cholesterol is the mobilization of lipoids into macrophages for the purpose of either esterification or emulsification. It has been pointed out by Kutschera-Aichbergen [12] and Lison [13] that decision as to the chemical nature of the lipoids from their staining properties is hazardous. From their indifference to polarized light one might believe that the fine sudan-staining droplets are for the most part fats and fatty acids.

The older lesions showed many intracellular optically active droplets, many of which exhibited the polarization cross. The presence of the latter droplets has led to the idea expressed by Basten [11] that esterification precedes phagocytosis. In these sections there were also many extracellular droplets showing the polarization cross, which possibly indicated extracellular esterification.

It was hoped that careful microscopic study would reveal some difference between the lesions of the thyroid test animals and those of the controls. This difference might be expected in the size and number of histiocytes and their degree of development into "foam cells." There also might be differences in the size, number and character of the giant cells. Finally the amount of optically active material left in the form of crystals could be readily compared in frozen sections in control and test animals. Careful comparison, however, revealed that none of these differences were present.

COMMENT

It may be objected that removal of pure cholesterol is not analogous to the removal of lipoids from the aorta, for this contains a large proportion of esters. It is possible that the removal of cholesterol has as one of its slower steps, esterification, which may be unaffected by the general metabolic activity of the phagocytic cells. Thannhauser and Magendanz [10] stated that xanthomas become relatively higher in free cholesterol with age, which indicates that esters may be removed more readily than free cholesterol. In the lesions examined, however, it is estimated that from one third to one half of the original crystalline material was changed into droplet form and taken up into phagocytic cells. If one may believe that cholesterol is well on its way to its

12. Kutschera-Aichbergen, H.: Virchows Arch. f. path. Anat. **256**:569, 1925.
13. Lison, L.: Bull. d'histol. appliq. à la physiol. **10**:237, 1933.

ultimate local fate by the time it is taken up into granular phagocytes, it is clear that thyroxin has had ample opportunity to exhibit its effects on these later stages of the removal process. The numerous extracellular droplets showing the polarization cross were present in almost the same numbers in the test and control lesions.

Thannhauser and Magendanz [10] pointed out that it is likely that cholesterol absorbed or synthesized in metabolic processes is excreted unchanged. This indicates that there is probably little cellular destruction of the cholesterol molecule. Such a conception fits well with the finding that thyroxin does not stimulate the removal process, for the effect of thyroxin is on cellular oxidation processes. The numerous very fine sudanophilic optically active droplets seen in frozen sections and the fine foam structure of the phagocytic cells in paraffin sections suggest that the essential process of removal is a purely physical one of emulsification and possibly solution in fine fat droplets.

While this material demonstrates clearly the conversion of cholesterol from crystalline form into intracellular droplet form, it does not throw much light on the ultimate removal of the cholesterol. Since the appearance of the crystals is so different from that of the optically active droplets and since the dispersion of the cholesterol influences its optical activity, it is impossible to estimate how much of the original deposit of cholesterol has been removed from the lesion.

Beidermann and Hoefer [14] showed that lipoid-laden macrophages retain the power of migration. Many foam cells were found some distance from the main deposit of cholesterol, but this was interpreted as being due to a dissemination of the injected material rather than to migration of macrophages. The only clue to the mechanism of removal suggested by this material is the finding that some of the older lesions showed large granular macrophages which appeared to have disintegrated and thus discharged their highly emulsified contents into the lymph spaces.

The observations described indicate that there is no marked and immediate effect exerted by thyroid on the removal or destruction of cholesterol. There may, of course, be some less marked, slower effect which can be detected only by quantitative measurement. In view of the absence of any thyroid stimulation of the removal of cholesterol by macrophages it seems that it would be more logical to seek the mechanism of this protection from atheroma in an effect on excretion or synthesis.

CONCLUSION

Thyroid feeding does not accelerate the removal of cholesterol from intracutaneous experimental deposits in the rabbit.

14. Biedermann, W., and Hoefer, K.: Arch. f. exper. Zellforsch. **10**:93, 1930.

Case Reports

PRIMARY FIBROSARCOMA OF THE BRAIN

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To the present time only 6 tumors composed of fibroblastic tissue have been reported as primary in the brain substance. Histologically they resembled fibroblastoma occurring elsewhere in the body, although their origin has been a moot question. It seems permissible to report another such tumor, not only because of the rarity of tumors of this type but also because of the additional evidence available as to their probable origin.

The reports of the first 4 primary fibroblastic tumors of the brain to be recorded in the literature have been reviewed by Baker and Adams [1] and will be summarized only briefly in this report. Bailey [2] reported 2 tumors of this type. One of these was studied by Mallory. The first tumor occurred in the right temporal lobe of a 42 year old woman as a semiopaque cartilaginous mass adherent to the dura. Histologically it was typical of fibrosarcoma, being composed of spindle-shaped cells separated by reticulin and collagen. Bailey's second tumor was an operative specimen removed from the right lateral ventricle of a 19 year old youth. It consisted of streams of spindle-shaped cells which had a delicate cytoplasm and oval or elongated nuclei containing dustlike chromatin material. Collagen was abundant in the degenerative areas.

Mallory [3] described a primary fibroblastic tumor in the right frontal lobe of a 33 year old man. It measured 5 cm. in diameter and was of an unusual firmness and whiteness. Histologically it was a slow-growing fibrosarcoma.

Alpers, Yaskin and Grant [4] added a fibroblastoma removed from the right frontotemporal region of a 52 year old man. It was an encapsulated white fibrous tumor, composed of loosely packed cells and myxomatous tissue. The rich intercellular substance consisted of fibrous tissue and fibroglia. Areas of degeneration and blood vessels were numerous. The neoplastic cells in close association with the walls of the blood vessels were in a stage of proliferation and were assumed to be "centers of growth" for the tumor.

Baker and Adams [1] reported a primary fibroblastoma found at autopsy in the right frontal lobe of a 10 year old girl. The tumor measured 5 cm. in all diameters and was extremely firm and sharply circumscribed but not encapsulated. On the cut surface it was white, semiopaque and almost gritty. It showed no attachment to the dura. The tumor was uniformly composed of fine and coarse strands of intertwining collagenous fibers that extended in all directions and stained readily with

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1. Baker, A. B., and Adams, J. T.: Am. J. Path. **13**:129, 1937.

2. Bailey, P.: Arch. Surg. **18**:1359, 1929.

3. Mallory, T. B.: New England J. Med. **203**:177, 1930.

4. Alpers, B. J.; Yaskin, J. C., and Grant, F. C.: Arch. Neurol. & Psychiat. **27**:270, 1932.

azocarmine. In some areas these intercellular fibers were fused to form homogeneous bundles, which had a hyaline appearance. In certain parts of these bands occurred plaquelike thickenings. Interspersed among the fibers of collagen were a moderate number of irregular cells. The majority were elongated and contained scanty cytoplasm with a finely granular nucleus. The brain tissue adjacent to the neoplasm showed extensive glial and microglial reaction, but within the tumor only microglia cells were present. Blood vessels were numerous in all sections but especially in the more cellular areas. These vessels varied from the more frequent endothelium-lined cavities to the less frequent vessels having walls typical of cerebral arteries. In no case, however, were there any proliferative areas comparable to the "centers of growth" described by Alpers, Yaskin and Grant.[4] Numerous hemorrhages were present, most of which were not perivascular, although there were a few so-called ring hemorrhages.

Meyer and Scheller[5] reported a fibromyxoma which protruded from the left temporal region of a 25 year old woman. This fluctuant mass had been obvious since a few months after birth, with no clinical symptoms until the age of 18. At autopsy the tumor was cystic and very large, replacing the greater part of the right parietal area. It extended from the frontal region to the transoccipital sulcus. The right side of the cerebrum and the basal structures were markedly distorted, and the convolutions were flattened. The tumor was not attached to the meninges. Externally the new growth appeared grayish and glassy, and was of a tough elastic consistency. Its cut surface contained numerous cysts of all sizes. The solid portions of the neoplasm were variable in appearance, some being whitish and calcified, while others were hemorrhagic, mucinous and soft. Microscopically, the tumor was composed of connective tissue septums interspersed with young fibroblasts. To the authors, the characteristic feature of this new growth was the presence of degenerative fibroblasts, which had become vacuolated and filled with a mucoid substance. The authors expressed the belief that these vacuoles ruptured, discharging their mucin, and were therefore the source of the mucin found in the interstices. The tumor was vascular, and from the adventitia of the vessels many fine collagenous strands extended outward into the adjacent tissue. This outgrowth of connective tissue from the adventitia of the blood vessels resembled the "centers of growth" described by Alpers, Grant and Yaskin.[4] The tumor both invaded and compressed the surrounding tissue. Other features observed were lipomas of the meninges and small cysts of the kidney.

REPORT OF A CASE

A white man aged 52 years had been somewhat uncooperative and careless for the past five years. One year prior to his admission to the hospital he began to complain of headache and periods of dizziness. He continued to work, however, until five months prior to his admission, at which time he suddenly suffered a convulsive seizure, associated with unconsciousness lasting for forty-five minutes. The patient had complete amnesia for this attack. Following this spell he remained at home for seven weeks, during which time he complained of weakness and malaise. The headache, which had left following the seizure, returned. His behavior throughout this time remained normal. The patient then returned to work, but it was noticed that his judgment was becoming poor. Two months prior to admission he became peculiarly quiet and began to stumble in his speech.

5. Meyer, H. H., and Scheller, H.: Virchows Arch. f. path. Anat. **300**:473, 1937.

He would express himself with the wrong words, although he seemed to know what he wanted to say. This defect in speech was not continuous but came and went, many days passing in which it was not present at all.

When first seen by a physician, he complained of a heavy feeling in his head. The results of an examination of the cranial nerves were negative. There was weakness of the right upper extremity and absence of the patellar reflex on the same side. The Chaddock, Oppenheim and Gordon signs were observed on the right. Incoordination was noticed in the performance of the right finger to nose test. Superficial and deep sensation were intact. The rest of the neurologic examination gave negative results. The patient presented mild motor aphasia, an apathetic emotional attitude and some diminution in attention. Laboratory studies gave negative results. Roentgen studies after injection of air into the lateral ventricles revealed them to be displaced to the right.

A few days after admission a craniotomy was performed and the left frontal lobe explored. No tumor was encountered. Subsequent to the operation hyperpyrexia and circulatory instability developed, and the patient died the following day.

Autopsy.—The brain revealed asymmetry due to an increase in the size of the frontal lobe of the left hemisphere. In this region there was an operative cavity, measuring 4 by 5 cm. Coronal sections of the left cerebral hemisphere revealed 2 tumors. One was near the frontal pole and the other in the temporal lobe (fig. 1 *A* and *B*). Both were of the same appearance and consistency but did not seem to be united at any point. On cut section they were hemorrhagic and necrotic, but not encapsulated, and had the characteristic gross appearance of gliomas.

The tumor in the frontal lobe was situated immediately beneath the operative cavity. It measured 3 by 2 cm. in the vertical plane and encroached on the inferior surface of the frontal lobe but was separated from the surface by a thin layer of cortex (fig. 1 *A*). The tumor extended posteriorly along the frontal horn of the lateral ventricle for a distance of 3 cm.

The other tumor, in the posterior part of the left temporal lobe, was likewise separated from the surface by a thin layer of cortex. Beneath the surface it measured 4 by 3 cm. and extended to a depth of 3 cm. (fig. 1 *B*). Both tumors merged imperceptibly into the surrounding brain tissue and were entirely intracerebral. There was no attachment to the dura.

The only abnormalities observed on cut section of the right cerebral hemisphere were generalized thinning of the cortex, numerous subependymal petechiae and a large clot filling the frontal pole of the lateral ventricle. There was no hydrocephalus. The basal structures and cerebellum were uninvolved.

Microscopic Appearance.—The tumors were similar in histologic appearance. For purposes of description they can be considered as divided into three zones—central, middle and peripheral. The large central portion was composed of a necrotic hemorrhagic material. In this area, thickened and degenerated walls marked the outline of many former blood vessels. The middle zone was made up of actively proliferating fibroblastic tissue and was very cellular, with an irregular arrangement (fig. 2 *A* and *B*). Intercellular collagenous fibers, which stained blue with the azocarmine, were numerous; they appeared in large sheets between the proliferating cells. Nerve and glial fibers, which showed degenerative changes, had been entrapped by this rapidly growing tissue. Blood vessels were conspicuous and appeared to be a part of the neoplastic process, if not the actual

source of it (fig. 2 *B*). The peripheral zone of the tumor represented the neuroglial reaction to the invasion of the fibroblastic elements. Here there was gradual blending of the reactive into the normal brain substance, with no sign of capsule formation.

The most characteristic part of each tumor was found in the middle zone, where one could study the variable structure of the proliferating tumor cells. The small spindle-shaped cells contained hyperchromatic nuclei. Their cytoplasm was abundant, streaming from either pole, and took a dull brick color. The intercellular fibers of such cells were few and were arranged in parallel fashion as though they

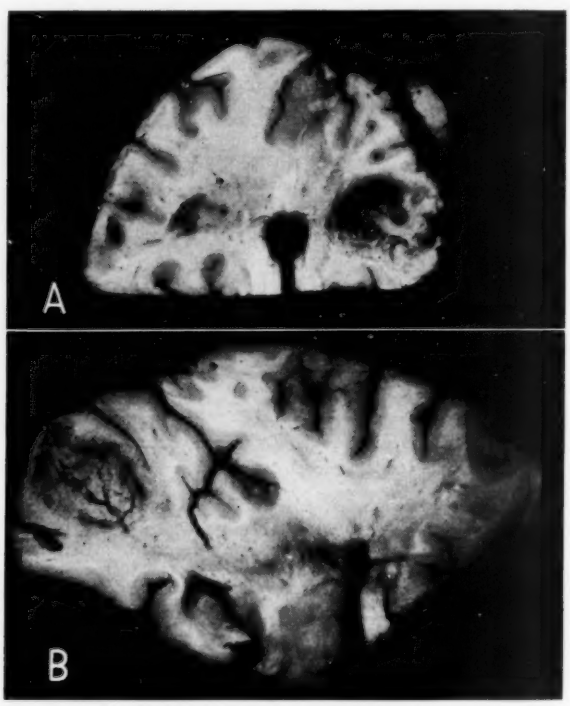

Fig. 1.—*A*, coronal section through the left frontal lobe showing the hemorrhagic, necrotic intracerebral tumor. *B*, section through the temporal neoplasm. This tumor is similar in appearance to *A* but is even less circumscribed and merges into the surrounding tissue.

had a definite objective. Mitotic figures were frequent in these areas (fig. 2 *A*). Other cells possessed large oval vesicular nuclei with a heavy nuclear membrane and clumped chromatin (fig. 2 *B*). Here the scanty cytoplasm was stellate. The fibers were more numerous and intertwined in aimless fashion to produce a reticular pattern. Intermediary forms, of course, were present. There was very little hyaline degeneration. Other cellular elements of connective tissue were also seen—macrophages, eosinophils and plasma cells.

The compressed and degenerated brain substance which lay adjacent to the tumors introduced a variety of cell reactions which slightly complicated the picture.

In this peripheral zone the gemistocytic astrocytes were more abundant than the pilocytic ones. These cells were made more conspicuous because of the partial demyelinization of the surrounding brain tissue. Microglia cells were interspersed with the other neuroglial elements but were most numerous in the perivascular

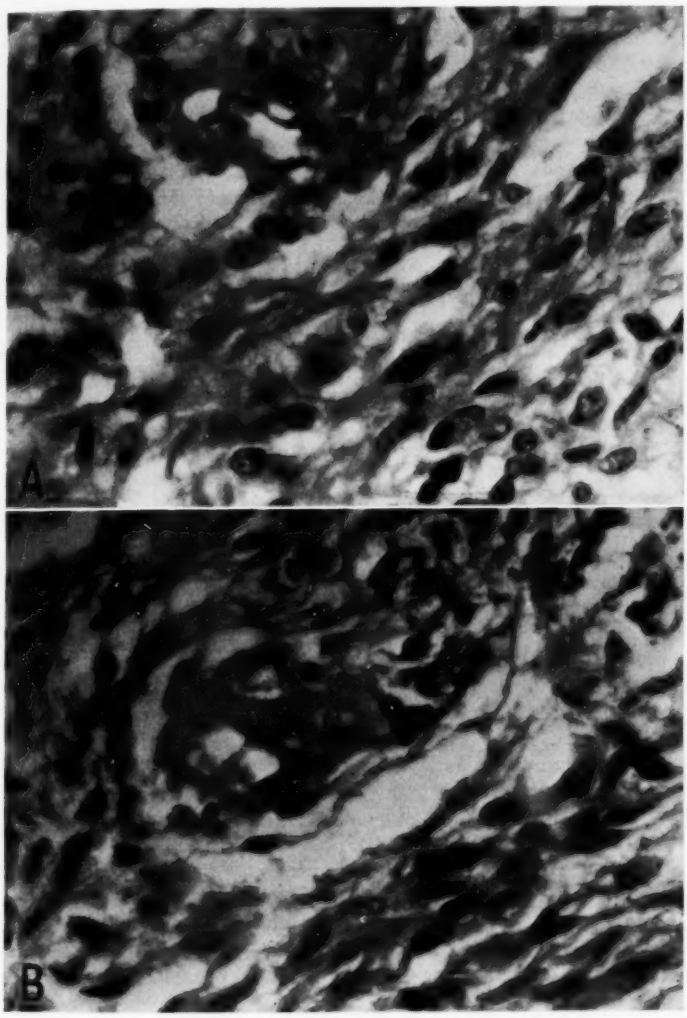

Fig. 2.—*A*, section through the cellular portion of the frontal tumor. Note the variable structure of the proliferating tumor cells. Mitoses are numerous. *B*, photomicrograph of the vicinity of a small blood vessel. The fibroblastic proliferation can be seen extending from the vicinity of the vascular adventitia into the surrounding tumor tissue.

spaces, where they had migrated with their ingested cerebral débris. Lymphocytes, polymorphonuclears and plasma cells could also be found in the perivascular regions, intermixed with the scavenger cells.

Throughout the tumors the blood vessels were increased in number, and their walls, in thickness. In the area of neuroglial reaction (peripheral zone) fibroblastic proliferation was seen in the outer layer of many of the vessel walls. Where the vessel consisted of a single layer of endothelium, a fibroblastic meshwork surrounded the vessel, enclosed the perivascular cells and extended into the adjacent brain tissue.

In the actively neoplastic zone one could observe large strands of connective tissue extending from the vicinity of the vascular adventitia into the surrounding tumor tissue (fig. 2*B*). This proliferating connective tissue had caused a distortion of the architecture of the vessels and a constriction of their lumens. The resultant thrombosis led to the necrosis which comprised the bulk of the tumor. The fibroblastic tissue which surrounded the still patent blood vessels was similar to that of the remainder of the tumor but was more dense and concentrically arranged. The density and nature of the fibroblastic growth prevented any perivascular hemorrhage around these vessels.

In summary, these neoplasms were considered to have had their origin in connective tissue because the type cell was the fibroblast. The neoplastic tissue was classed as sarcoma because of its invasive properties and because of the evidence of rapid growth. There was no sign of capsule formation. Many areas contained spindle-shaped cells with hyperchromatic nuclei and abundant cytoplasm. Intercellular substance was scanty in these areas, and mitotic figures were common. Centers of growth around blood vessels were a characteristic feature.

COMMENT

The 7 primary brain tumors of fibroblastic origin can be subdivided into 4 fibrosarcomas, 2 fibroblastomas and 1 fibromyxoma. They occurred in the frontal and temporal regions in patients ranging in age from birth to the age of 52 years. One tumor had a connection with the meninges.

Such tumors may have as their origin the pia, the vascular adventitia or the pia surrounding the blood vessels. Alpers, Yaskin and Grant [4] stated, "There can be little doubt . . . that around the many vessels in our tumor, were tumor cells that arrange themselves in intimate relations to the vessels. They seem to be part and parcel of vessel wall." These authors suggested as an etiologic possibility those cells which, like the capillaries, are derived from embryonic connective tissue and which accompany the capillaries. The cells which accompany the capillaries were formerly called perithelium, but according to Maximow [6] this term should be discarded as it included several types of cells. He believed that as the vessel becomes more complex the adventitia gradually merges with this cellular element and that undoubtedly both have a common source. Alpers, Yaskin and Grant [4] concluded that the fibroblastic origin is from the leptomeninges, cerebral vessels or cells associated with vessels capable of differentiating into fibroblastic cells.

Meyer and Scheller [5] offered, as the source of their fibromyxoma, mesenchymal rests or the adventitia of the blood vessels of the brain. The deformtiy of the skull since birth and the concomitant existence of lipomas of the leptomeninges and cysts of the kidney suggest the mesenchymal rest.

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6. Maximow, A. A., and Bloom, W.: A Textbook of Histology, ed. 2, Philadelphia, W. B. Saunders Company, 1934, p. 245.

Although the derivation of the leptomeninges is still unsolved, it is known that they become invaginated to form the perivascular spaces of Virchow and Robin. Because of this close proximity to the vascular adventitia it is difficult to determine absolutely whether these tumor cells originate from the leptomeninges or from the vascular adventitia. In the present case tumor cells were found around capillaries and could well be derived from the undifferentiated mesenchymal cells. In vessels having more complex walls the proliferation apparently was occurring in the adventitia and spreading peripherally. The histologic evidence certainly favors the opinion that the tumor cells were arising from the connective tissue of the adventitia or from its precursor, the less differentiated mesenchymal cells.

SUMMARY

A case is reported in which 2 separate fibrosarcomas were found in the left cerebral hemisphere of a 52 year old man.

From the histologic studies of fibroblastic tumors of the brain it appears that their most probable origin is from the connective tissue of the adventitia or from its precursor, the less differentiated mesenchymal cells.

HEMOBLASTIC SARCOMA (PRIMITIVE RED CELL TYPE) FOLLOWING POLYCYTHEMIA VERA

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The case to be presented is a remarkable instance of overcompensation of hemoblastic tissue following the exhaustion of abnormal erythropoiesis, which was terminated by vigorous benzene and roentgen ray treatment.

REPORT OF CASE

A woman of 47 years was admitted to the hospital April 26, 1936. Seventeen years earlier, at the age of 30, her red cell count was 11,000,000, and a diagnosis of polycythemia vera was made. She was treated during a period of thirteen years with roentgen radiation and benzene. The treatment was discontinued in 1932, when the hemoglobin content dropped to 70 per cent and the red cells to 3,500,000. Five years prior to admission she had a series of attacks of chills and fever associated with herpes zoster and Horner's syndrome on the left side. These attacks were never explained. During the two years prior to admission her symptoms had been largely those of progressive severe anemia. She had been given repeated transfusions of blood and parenteral injections of liver. Six months prior to admission she began to have painful swelling in the region of the right scapula, which on biopsy was diagnosed as sarcoma of the scapula. This mass almost disappeared with roentgen therapy. A pleural effusion occurred on the left side, and a smear of sediment from the fluid revealed 23 per cent myeloblasts. At this time a count made on the peripheral blood showed, in addition to the secondary anemia, 14 per cent myeloblasts and 6 per cent normoblasts.

During the first two months of the patient's stay in the hospital numerous lumps developed over the jaw, sternum, abdomen and groin. They were painful and tender. These masses appeared at sites of injection or of injury.

On examination during her stay in the hospital, she presented an enlarged liver, a hard, firm enlarged spleen, an inconstant soft systolic murmur at the apex of the heart, signs of consolidation at the base of the left lung and an old unhealed abscess of the left buttock. In addition, masses of soft tissue were felt at the angle of the jaw on the left, over the right temporal region, at the left corner of the mouth, over the sternum, in the abdominal wall and in the right and left groins. The inguinal and the anterior cervical lymph nodes were enlarged, some being as large as a lemon.

The red blood cell count was 3,000,000; the hemoglobin content, 58 per cent; the color index, about 1. The white blood cell count was 3,100, with differential percentages as follows: segmental polymorphonuclears, 44; staff polymorphonuclears, 5; myelocytes, 2; myeloblasts, 22; lymphocytes, 19, and monocytes 7. The platelet count was 75,000. The bleeding, coagulation and clot retraction times were normal. Chemical examination of the blood revealed a low level of

From the Laboratory Division, Montefiore Hospital.

cholesterol, 125 mg.; calcium, 12 mg.; phosphorus, 3.7 mg.; protein, 4.1 Gm.; albumin, 2.2 Gm.; globulin, 1.9 Gm.; icterus index, 4; bilirubin, 0.3 mg.; sugar, 98 mg.; urea nitrogen 12 mg. The tourniquet test was negative. The blood plasma volume was slightly increased.

The gastric juice revealed achlorhydria even after administration of histamine. Bence Jones protein was not present in the urine. The basal metabolic rate was + 64 and + 57 per cent. The Wassermann and Kahn reactions were negative.

Roentgen examination revealed pleural masses, especially over the upper lobe of the left lung, and increase in the width of the superior mediastinum. A large mass of soft tissue was also seen involving the sternum. There was slight osteoporosis of the distal ends of the shaft of both femurs and of the lower third of the right tibia, but it was not characteristic of a destructive lesion.

Biopsy of the soft tissue mass in the abdominal wall revealed an appearance of chronic granulomatous tissue, which at the time was considered to be suggestive of Hodgkin's disease. The clinical diagnosis was myelosarcoma with the blood changes of myeloblastic leukemia.

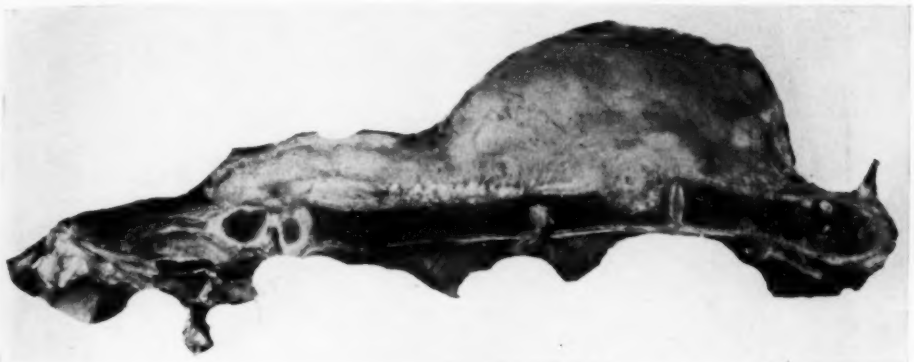

Fig. 1.—Cross section through the sternal mass.

The patient was extremely weak and declined rapidly. She died on June 1, five weeks after admission.

Autopsy.—The anatomic diagnosis was: hemoblastic sarcoma of a primitive red cell type, with invasion of subcutaneous and soft tissues, muscles, right femur, sternum, intercostal tissue, on the left vertebral column, pleura, lungs, left side of the diaphragm, heart, right kidney, gastrointestinal tract, left inguinal nodes, tonsils and uterus; diffuse hemosiderosis; splenomegaly; indeterminate endocarditis of the mitral valve, and an unhealed gluteal abscess on the left.

A large tumor mass, 9 by 6.5 by 5 cm., was present over the greater portion of the sternum. This mass was firmly adherent to the overlying skin and also to the underlying bone. On section it revealed erosion of the bony structure. It was partly necrotic but generally firm and whitish pink. Subcutaneous nodules were encountered in the scalp and left cheek, over both mandibles, in the right side of the face just anterior to the ear and in the left breast. They varied in diameter from 1 to 6 cm. On section they resembled the mass over the sternum. An abscess was present in the wall of the right upper quadrant of the abdomen. On section an extensive hemorrhage was encountered in the surrounding muscle

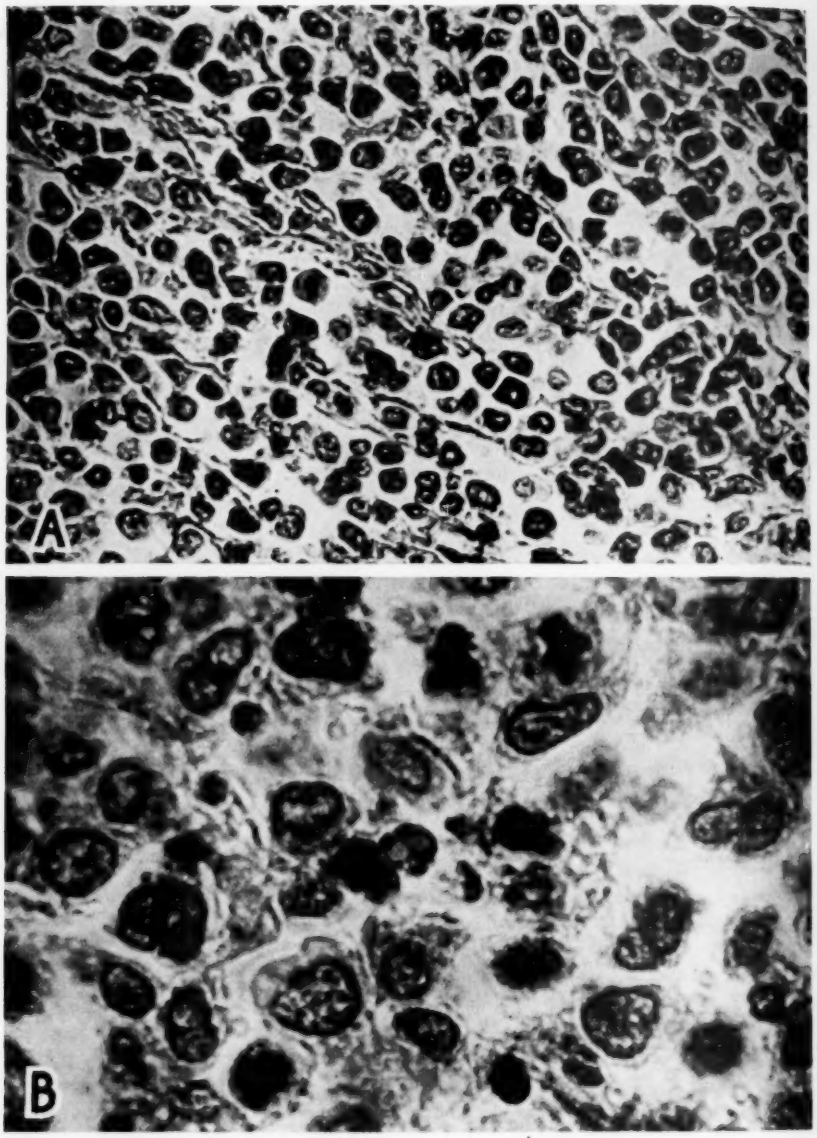

Fig. 2.—Microscopic appearance of the sternal tumor mass. Note the large numbers of stem cells. *A*, × 240; *B*, × 1,200.

tissue. A large abscess mass in the buttock contained tumor tissue in the surrounding muscle. A series of matted firm enlarged lymph glands filled the entire right axilla and extended down to the scapula. There were similar nodes in the left inguinal region. Tumor tissue infiltrated all the anterior mediastinal soft tissues and both tonsils.

The major vessels coming from the arch of the aorta were surrounded by tumor tissue. The left pleural cavity was obliterated by adhesions and extensively infiltrated by tumor, particularly at the base. The major portion of the left parietal pleura and the diaphragm were infiltrated and studded throughout with firm pink-white tumor nodules. On section the right lung contained one firm area of tumor tissue close to the hilus. Subpleural nodules were present throughout both lungs.

The spleen was enlarged (725 Gm.) and firm. On section the surface appeared somewhat glazed and pinkish red. The markings were not distinct. There was a small nodule, partly necrotic, beneath the capsule.

In the fundus of the stomach, along the greater curvature, was a large raised circular nodule, 5 by 5 by 1 cm., which on section contained pink-white tissue. Similar nodules were found beneath the mucosa of the cecum, colon and rectum, also in the substance of the right kidney and in the body of the uterus. They varied in diameter from 1 to 4 cm. The paravertebral muscles and soft tissue were infiltrated by tumor. The marrow of the vertebrae was deep red and hyperplastic. The thoracic vertebrae showed areas of firm pink-white tumor. The marrow of the right femur was completely replaced by tumor.

Microscopic Examination.—The tumor nodules and bone marrow were similar in appearance. In the marrow of the right femur the cells were arranged in solid sheets, which were apparently confluent hemopoietic nodules, the most primitive cells of which were centrally located. The large predominant cell measured about 20 microns in diameter, was rounded or oval and contained nongranular basophilic cytoplasm, occasionally vacuolated. The cell membrane was indistinct. The large nucleus, rounded or oval, was frequently notched in kidney bean fashion; it filled most of the area of the cell and was slightly eccentric. The nuclear membrane was coarse and sharply defined. Many dense nucleolar bodies were irregularly scattered throughout the nucleus. The nucleus was frequently seen in mitotic division. These cells were interpreted as hemocytoblasts.

More peripherally there were slightly smaller spherical cells with round, regular nuclei and more or less evenly distributed nucleoli, suggestive of proerythroblasts and erythroblasts. Adjacent to these were many smaller cells, about 9 microns in diameter, with small round nuclei containing dense chromatin material. The cytoplasm was basophilic in some and esosinophilic in others. The appearance was characteristic of normoblasts in various stages of development.

Occasional large cells, similar in appearance to the hemocytoblasts, contained eosinophilic granules in their cytoplasm; some revealed horseshoe-shaped nuclei. These were interpreted as promyelocytes and myelocytes.

Some cells, about twice the size of the hemocytoblasts, with irregular cytoplasmic projections and large dense irregular nuclei, appeared to have the characteristics of megakaryocytes.

The mass in the sternum, as well as other masses, consisted of primitive hematopoietic elements, chiefly hemocytoblasts and many erythroblasts and normoblasts, arranged in a fashion similar to that seen in the bone marrow. Hemorrhage, necrosis and old blood pigment were scattered throughout.

COMMENT

This condition was interpreted as a stem cell sarcoma of a primitive red cell type. It was an interesting example of overcompensation following exhaustion of erythropoiesis by benzene and roentgen therapy, instituted for the treatment of polycythemia vera. The hemoblastic sarcoma of a primitive red cell type may be looked on as a phase of overcompensation following this exhaustion and may be operative through a central mechanism. So-called myelogenous leukemia has been known to occur as a late phase in cases of exhausted polycythemia vera, but this seems to be the only instance on record, to our knowledge, of a tumor of a primitive red cell type in a human being.

BALL THROMBUS OF THE HEART

Report of a Case with Review of the Literature

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Up to 1924 Abramson [1] was able to collect from the literature only 22 cases of ball thrombus of the heart, to which he added the twenty-third. His article is excellent and should be read by every one interested in this condition. Since then, other cases have been reported,[2] bringing the total to 30 cases. Ball thrombus of the heart is therefore of sufficient rarity to warrant report of another case.

As the term is used in this paper, a ball thrombus must fulfil the criteria set down by Welch;[3] i. e., there must be (*a*) entire absence of attachment, with consequent free motility; (*b*) imprisonment in consequence of an excess in the diameter of the thrombus over that of the first narrowing in the circulatory passage ahead of it, and (*c*) such consistency and shape that the thrombus will not of necessity lodge as an embolus in the passage. Separation of cases of this particular type of thrombus into a group is of somewhat academic interest since, clinically, similar symptoms may be produced by a pedunculated thrombus [4] of the heart, as well as by a large vegetation of subacute bacterial endocarditis protruding into the mitral orifice (Schiller;[5] Schwartz and Biloon [2e]). However, these cases do seem to fall into a group by themselves, and probably the condition deserves to be considered as a pathologic entity.

REPORT OF CASE

A white woman 43 years of age was admitted Aug. 18, 1937, complaining of spells of vomiting after meals. The attack for which she was admitted had begun

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From the Pathologic Laboratory of Providence Hospital.

1. Abramson, J. L.: Ann. Clin. Med. **3**:327, 1924.

2. (*a*) Cleland, J. B.: M. J. Australia **2**:50, 1936. (*b*) Covey, G. S.; Cook, R., and Rogers, F. L.: Am. J. M. Sc. **175**:60, 1928. (*c*) Elson, J.: Am. Heart J. **10**:120, 1934. (*d*) Potter, E. B.: Ann. Clin. Med. **4**:736, 1936. (*e*) Schwartz, S. P., and Biloon, S.: Am. Heart J. **7**:84, 1931.

3. Welch, W. H., in Allbutt, F. C.: A System of Medicine, London, Macmillan & Co., 1899, vol. 6, p. 185.

4. Kaplan, D., and Hollingsworth, E. W.: J. A. M. A. **105**:1264, 1935.

5. Schiller, I. A.: J. Mt. Sinai Hosp. **2**:153, 1935.

two weeks previously. Nausea did not always precede these attacks. There was no pain associated with the vomiting at any time. Such spells had occurred at intervals of several weeks to a year for the past five years, each lasting about a week. One month before admission a cough developed, with expectoration, which gradually lessened until it disappeared two weeks before admission. There had been a loss of 20 pounds (9.1 Kg.) in the past two years. During the past few years there had been irregular attacks of asthma at any time of the year, which the patient associated with taking cold. The menstrual history was entirely normal. The patient had a child, living and well, 17 years old. The past medical history revealed: chickenpox, pertussis, measles and diphtheria. The family history was negative.

The patient was a rather emaciated white woman, who did not appear acutely ill. The head and neck were normal. The eyes reacted to light and accommodation. The throat was normal. The teeth were in fair condition, with several missing. The lungs were clear. As regards the heart, there was splitting of the first sound with frequent extrasystoles. There was slight enlargement of the heart. There was a systolic murmur over the entire precordium. A faint presystolic or a late diastolic murmur was also believed to be present. The blood pressure was 120 systolic and 80 diastolic; the pulse rate, 68. The abdomen was somewhat retracted, and the liver was considerably enlarged but not tender. The diagnosis was rheumatic valvulitis with mitral stenosis and chronic gastritis.

The hemoglobin content was 68 per cent (Dare); the red blood cell count, 3,500,000; the white blood cell count, 11,000, with 87 per cent polymorphonuclears, 11 per cent large lymphocytes and 2 per cent small lymphocytes. The Wassermann and Kahn tests were negative. The Van den Bergh direct reaction was negative; the indirect reaction, less than 0.3 mg. per hundred cubic centimeters.

Ten days after admission, the pulse rate began to rise and remained high, varying between 90 and 130 per minute. During the first five or six days the patient was slightly febrile, the temperature never rising over 100 F. After this, but for one slight rise to 99.6 F. on the thirteenth day of illness the temperature remained normal until the last nine days of life, when it became subnormal. The patient became progressively weaker; transfusion did not alter the course of the illness. September 15 the patient died, after two days of marked dyspnea and cyanosis of the lips and face.

Postmortem Examination.—The body was that of a white woman in the fifth decade of life, small of stature, fairly well developed and showing emaciation of grade 3. The pupils were equal and slightly dilated. The abdomen was distended. The subcutaneous fat was fairly well preserved. The margin of the liver was 10 cm. below the ribs in the right midclavicular line and 20 cm. below the xiphoid process, being displaced downward by a collection of fluid in the right pleural cavity. The right pleural cavity was filled with a slightly cloudy olive green fluid, estimated at 1.5 liters.

The heart was enlarged, especially to the left, the apex reaching the midaxillary line. The surface of the heart was smooth and glistening throughout. The tricuspid orifice admitted four fingers. The right ventricle showed definite hypertrophy. In handling the heart, before the left side was opened, a ball-like structure slipped out of the left atrium through the opening of an especially large pulmonary vein (fig. 1 *A* and *B*). This mass was almost perfectly round, 3.5 cm. in diameter, and showed no evidence of a pedicle or of a previous site of attachment. Its surface was covered with pinpoint-sized elevations, resembling "goose pimples," and the color of the mass varied from yellowish to deep rose.

It had evidently been lying within the left atrium as a free body. When the left atrium was opened, two masses of similar tissue were seen adhering flatly to the inner surface of this chamber, close to the mitral orifice (fig. 2). These masses were both irregular in shape; one was slightly triangular, measuring 2 by 1.5 by 1.5 cm. and having a thickness of about 1 mm., and the other was rather square, measuring 2.5 by 2.5 cm. and having a thickness of 2 mm. The latter could be easily dislodged. Both of these structures were covered by the same pinpoint-like elevations as the ball thrombus. The mitral valve was the seat of marked chronic fibrosis, due to an old rheumatic infection, which had resulted in a typical

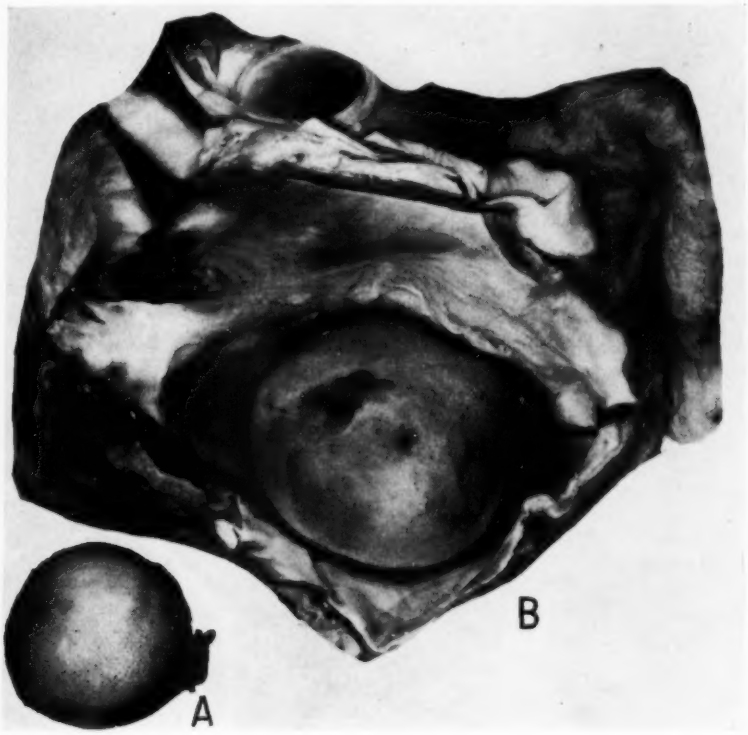

Fig. 1.—*A*, ball thrombus: Note the small pimple-like elevations. The rough area on the side is not a pedicle but an area in which the superficial layer of fibrin has become loosened from the mass. (The photographs are presented by permission of Lieut. Col. J. E. Ash, curator of the United States Army Medical Museum.) *B*, ball thrombus in situ, showing how completely the thrombus could fill the mitral orifice.

funnel-shaped valve that would not admit the tip of a finger. The greatest diameter of the opening was about 1 cm. (fig. 2). The aortic valve was normal, but the aorta itself was markedly hypoplastic, admitting only the little finger. The left ventricle showed no hypertrophy and seemed rather small.

The right lung was somewhat compressed by the collection of fluid referred to. However, on section the tissue was pale pink and crepitant throughout. The left

lung showed in the lower portion of the lower lobe a rather large area of increased density, which did not crepitate. On section this tissue showed a pale red area which was irregular, depressed and surrounded by very firm and moderately congested tissue. The upper lobe was crepitant throughout and on section was pale pink.

The spleen was bound down by dense adhesions. It was slightly enlarged, and the lower two thirds was the seat of an extensive degenerated infarct. The

Fig. 2.—Mitral valve. There is marked stenosis. The slitlike orifice is not clearly seen but was about 1 cm. in length.

upper pole contained another infarction, which was small, close to the periphery and surrounded by firm and markedly congested tissue.

The liver was normal in size. On section it showed the characteristic nutmeg appearance and was congested (grade 3). The gallbladder was considerably thickened and contained a small amount of dark green bile. There were no stones. The pancreas was normal in size but showed slight congestion. The adrenals were slightly pale but otherwise normal.

The left kidney was normal in size (175 Gm.). On section the cortex was of average thickness. The medulla showed numerous small cystic excavations. The outermost portion of the cortex showed a peculiar deposit arranged in linear

streaking, suggestive of calcium deposits. The right kidney was replaced by a small cystic mass, 3 cm. in greatest diameter, which on section showed two small thin-walled cysts filled with clear fluid.

The uterus was atrophic and contained no tumors. The fallopian tubes were thin walled and congested. The ovaries were markedly calcified and slightly increased in size.

Pathologic Diagnosis.—The following conditions were diagnosed: rheumatic heart disease with marked mitral stenosis; ball thrombus of the left atrium; cystic degeneration of the right kidney; splenic infarction; pneumonitis of the lower lobe of the left lung; chronic cholecystitis; sclerosis of the ovaries.

Microscopic Examination.—The examination showed slight calcium deposits in the renal tubules, fibrosis of the lungs with exudation of leukocytes into many alveoli and a few areas of perivascular fibrosis in the heart. The thrombus was not sectioned, as it was desired to preserve it intact. In previous cases section showed the thrombus to be composed of layers of fibrin, at times with a gelatinous core.

A review of the literature reveals the following facts in relation to cases of this type: Of the 21 cases in which sex was recorded, 17 concerned females and 4 males. This is in keeping with the sex incidence of rheumatic infection in general. The reported ages of the patients ranged from 15 to 55 years. Among the females, the highest frequency was in the fifth decade. Among males, the age of greatest frequency varied from 16 to 28 years. The ball thrombus therefore caused earlier death in the male than in the female, probably because of the less sheltered life of the male. In every case mitral stenosis was present, and in most cases, to a marked degree. The ball thrombus was found in the left atrium in all but a single case.[1] In this case it was found in the right ventricle.

The most universal symptom was dyspnea, and in every case it was prominent. In most cases the shortness of breath was extreme, and in many cases it was the presenting symptom. Embolic phenomena were fairly frequent. In those cases in which an antemortem diagnosis was made (or considered) the most prominent clue to the diagnosis, according to Abramson,[1] was the transient interference with the peripheral circulation. This was stressed greatly by Battistini and by Bozzolo, who diagnosed the condition clinically, although in their particular cases the cardiac thrombi were pedunculated. This, however, does not destroy the value of their findings, as the signs and symptoms would be the same whether the thrombus was free or attached. In many cases a cold and mottled extremity, cadaveric in appearance, became entirely normal within twenty-four hours, or a pulse that was faintly perceptible or imperceptible returned to normal. Another rather frequent embolic phenomenon met with was hemiplegia occurring within one or two years of the fatal outcome. This was seen in 5 cases.[1]

Apparently the rheumatic infection responsible for the mitral stenosis is responsible for the formation of the ball thrombus also. In many cases an area of localized thickening was found in the endocardium of the left atrium. This was an irregular elevated patch, rather densely adherent, which could easily have been the site of origin of the nucleus

of the ball thrombus. After separation from this site of origin, the thrombus could become spherical and coated with a smooth laminated covering of fibrin.

In reviewing the cases in the literature as to symptoms and diagnosis, we have included certain cases in which a typical ball thrombus was not present but a thrombus that simulated the ball type clinically. Such instances are those of pedunculated thrombus of the left atrium and also those of certain large vegetations seen in subacute bacterial endocarditis involving the mitral valve. Incidentally, one of the interesting features of ball thrombi is that they have thus far appeared *only* in association with mitral stenosis, while pedunculated thrombi have occurred with other conditions, as hypertension. Of all the thrombi reported, i. e., ball thrombi, pedunculated thrombi and large occluding vegetations, only 11 have been diagnosed clinically, while of the 30 ball thrombi only 4 have been diagnosed clinically. However, the diagnosis is becoming more frequent, most of the thrombi of this type which have been recorded having been diagnosed in the last decade.

So, in spite of the infrequency of the diagnosis in the past, in certain cases the possibility that this condition is present should be at least suspected. Thus, according to Battistini, quoted by Abramson,[1] "the diagnosis of thrombi of the left auricle is possible as formed by the symptomatology and reconstructed by my two cases, that is: (*a*) signs of mitral stenosis; (*b*) disturbance of the general circulation; (*c*) entire debility of the pulse; (*d*) presence of gangrene of the lower extremities; and also the practical importance of the pulse in the radial and other accessible peripheral arteries." Later Elson,[2e] in presenting a new case, suspected before death, restated these criteria for the diagnosis as follows: ". . . diagnosis is frequently impossible, but it can sometimes be made on the basis of (*a*) long-standing mitral stenosis usually with auricular fibrillation, and (*b*) widespread and transitory disturbances in the peripheral circulation." In our case no suggestive symptoms were noted.

SUMMARY

Another case of ball thrombus of the left atrium is reported, with a review of the literature, showing this to be the thirty-first case reported. The condition is found only in association with long-standing mitral stenosis. It may be suspected in a case of long-standing mitral stenosis with or without auricular fibrillation when widespread and transitory disturbances in the peripheral circulation are found. Among the latter, a cadaveric coldness of the extremities is prominently mentioned.

General Reviews

COMPLEX INFECTIONS

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Until quite recently each infectious disease was generally believed to have a single and entirely specific etiologic agent. In fact, such a conception formed the basis of Koch's postulates for the proof of the causal relationship of an infectious agent to a definite disease. Rivers [1] has on several occasions pointed out that Koch's postulates could not be strictly applied to diseases caused by filtrable viruses, though even here the spirit of the postulates could be fulfilled easily enough. Rivers has furthermore called attention to the fact that in certain diseases of complex etiologic background the rules set down by Koch were not applicable. It is with this group of diseases of complex cause that I wish to deal.

My reasons for choosing such a subject were twofold. First, it seemed that the topic might lend itself well to pointing out the fallacy of thinking of infectious diseases in the old terms of Koch's postulates as invariably due to single agents. And second, the diseases to be considered are probably largely unfamiliar to those whose main interests lie in human medicine. Thus the discussion should at least avoid the repetition of already familiar knowledge.

Throughout the present paper the expression "complex infection" will be used to denote an infectious disease in which more than one agent plays an essential causal role; the expression "etiologic complex," to denote collectively the agents causally involved. Only those diseases that have been reasonably proved to be of complex cause will be discussed. These bear neither relation nor similarity to what are commonly known in medicine as mixed or complicated infections: all occur under natural conditions as definite clinical entities.

The story of the development of knowledge concerning the complex infections is as exciting and intriguing as a detective yarn and equally full of fortuitous observations, obscure deductions and retrospective simplicity. In narrating this story I intend to consider each disease indi-

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1. Rivers, T. M.: J. A. M. A. **107**:206, 1936; J. Bact. **33**:1, 1937.

vidually and to discuss the various observations and clues leading to its detection as a complex infection, together with the theories as to how the paired causative agents act in producing the disease. To facilitate understanding of the plan of presentation to be followed, the complex infections of which I shall speak are listed here, with their causative agents.

Disease	Causative Agents
1. Blackhead of turkeys	Histomonas meleagridis + Heterakis gallinae
2. Rugose mosaic of potato	Mottle (X) virus + vein-banding (Y) virus
3. Streak of tomato	Mottle (X) virus + tobacco mosaic virus
4. Swine influenza	Haemophilus influenzae suis + virus of swine influenza
5. Type III coryza of fowl	Haemophilus gallinarum + coccobacilliform bodies
6. Tulip breaking	Tulip virus 1 + tulip virus 2
7. Infectious myxomatosis of rabbits	Fibroma virus + Berry-Dedrick factor

It is evident that these diseases are diverse both as to hosts and clinical types. Furthermore, the etiologic agents are a heterogeneous collection, ranging from viruses through bacteria to protozoa and worms.

BLACKHEAD OF TURKEYS

Blackhead has been defined as an infectious disease of turkeys and, to a lesser extent, of chickens, caused by Histomonas meleagridis. The pathologic alterations, which are limited mainly to the ceca and liver, are those of caseous necrosis. At autopsy the livers of affected birds are found to be markedly enlarged and spotted with multiple focal areas of necrosis ranging from pinpoint size to a centimeter or more in diameter. The cecal walls are thickened, and the cecal lumens are filled by plugs of necrotic caseous material. The mucosa and submucosa are inflamed or necrotic and desquamated. Histomonads are plentifully present in the cecal and hepatic lesions.

The disease was first seriously investigated by Theobald Smith, and in 1895 he described as the cause a protozoan parasite, since called Histomonas meleagridis.[2] The initial claim of this parasite to consideration as the etiologic agent was based entirely on the fact that it was uniformly present in the lesions.

2. Smith, T.: Investigations Concerning Infectious Diseases Among Poultry, Bulletin 8, United States Department of Agriculture, Bureau of Animal Industry, 1895.

In later years Smith and Graybill became interested in the mode of spread of the disease. There were a number of epidemiologic facts not clearly explicable on the basis of a pure histomonad infection. For instance, turkeys fed feces containing Histomonas meleagridis from infected or recovered birds not infrequently failed to acquire blackhead. Furthermore, though the histomonads were known to be relatively fragile parasites and to be incapable of survival for long in material discharged from the ceca, pens in which infected turkeys had been kept remained infective for young turkeys long after any histomonad should still survive. Because of such epidemiologic discrepancies Graybill and Smith suspected that an accessory agent might be concerned either in the transmission of Histomonas meleagridis or in favoring its invasion and multiplication in the turkey. In harmony with this hypothesis they introduced into their experiments the common cecal worm of poultry, Heterakis gallinae, as the possible associated factor.[3]

Young disease-free turkeys were fed feces from old turkeys of a flock known to be infected, and with these feces were mixed cultures of embryonated ova of Heterakis gallinae. Blackhead developed in the young turkeys and all died. In a subsequent experiment Graybill and Smith included four groups of young turkeys. One group was fed turkey feces alone, one group was fed turkey feces mixed with embryonated ova of Heterakis gallinae, a third group received ova alone, and the fourth group served as unfed controls. No clinically recognizable blackhead appeared in the control group or in the group fed turkey feces alone. In the two other groups, however, typical blackhead developed in all the birds. From this experiment it appeared that blackhead could be produced in turkeys merely by feeding them embryonated ova of Heterakis gallinae. Further experiments both with turkeys and chickens confirmed this observation. However, though the disease had apparently been induced by embryonated ova of Heterakis alone, at autopsy the lesions of the experimental birds were shown to contain Histomonas meleagridis. These observations led Graybill and Smith to the assumption that either their experimental turkeys and chickens had been healthy carriers of histomonads or their cultures of Heterakis gallinae had been contaminated by these parasites. They did not definitely choose between the two possibilities, though they favored the latter. At any event, they visualized the blackhead produced as resulting from the concerted activity of Histomonas meleagridis and Heterakis gallinae. Supposedly, the larvae of Heterakis gallinae prepared the way for the destructive invasion of the walls of the ceca and the liver by Histomonas meleagridis.

3. Graybill, H. W., and Smith, T.: J. Exper. Med. **31**:647, 1920. Smith, T., and Graybill, H. W.: ibid. **32**:143, 1920.

But it remained for Tyzzer [4a] and Tyzzer and Fabyan [4c] to demonstrate the actual cooperation by which Histomonas meleagridis and Heterakis gallinae produce blackhead. They experienced no difficulty in confirming the correctness of the observation of Graybill and Smith that embryonated ova of Heterakis gallinae frequently produce blackhead when fed to young disease-free turkeys. Furthermore, by using great care in hatching and rearing their experimental birds they procured young turkeys that were undoubtedly not carriers of Histomonas meleagridis. In such birds the embryonated ova of Heterakis gallinae still produced blackhead, and histomonads were present in the lesions. This made it seem quite clear that the ova had been contaminated by histomonads, but it did not indicate whether the contaminating protozoans were within the worm eggs or whether they merely adhered to the surfaces of the eggs. The question was finally answered by some extremely ingenious experiments. Instead of incubating the ova of Heterakis gallinae in the usual way, in salt solution, the cultures were prepared in 1.5 per cent nitric acid. This concentration of acid was sufficient to kill any adherent histomonads quite promptly, and such cultures after three days proved even bacteriologically sterile. It was clear that the procedure adequately eliminated histomonads that were lying external to the shells of the worm eggs. The ova were kept in the acid until they could be observed with the microscope to have become embryonated. They were then fed to young histomonad-free turkeys. Blackhead developed, and histomonads were observed in the lesions.

Even more conclusive were the results obtained by feeding samples of heterakid material, some before and some after the eggs had become embryonated and hence hatchable. In these experiments the nonembryonated ova failed to cause blackhead, while the embryonated ones induced the disease. Such results indicated that infection following the feeding of heterakid material was dependent on the hatching of the eggs of the worm in the alimentary tract of the bird. Strangely enough, Tyzzer was never able to see histomonads microscopically in demonstrably infective heterakid eggs though they have been seen in the gut of the newly hatched larva.

From the observations just summarized it seems clear that Histomonas meleagridis is carried within the eggs of Heterakis gallinae and persists in the eggs during embryonation even in 1.5 per cent nitric acid. When the embryonated ova hatch in the alimentary tracts of the turkeys to which they have been fed, the young larvae penetrate the epithelium of the cecal glands, doubtless carrying their histomonads with them. Here they increase in size, damaging and separating the epithelium by

4. (*a*) Tyzzer, E. E.: Proc. Soc. Exper. Biol. & Med. **23**:708, 1925-1926; (*b*) Proc. Am. Acad. Arts & Sc. **69**:189, 1934. (*c*) Tyzzer, E. E., and Fabyan, M.: J. Exper. Med. **35**:791, 1922.

pressure and eventually producing an inflammatory reaction. Heterakis gallinae thus not only serves as the intermediate host for Histomonas meleagridis but also provides it with a portal of entry by damaging tissues and preparing them for parasitization by this protozoan. It seems to me that in blackhead one has a rather good example of cooperative action by two agents in producing a disease: a truly complex infection.

Some objection to the classification of blackhead as a complex infection might be raised on the ground that, as Tyzzer and his co-workers showed, the disease can be produced by Histomonas meleagridis alone if the histomonads are actually inoculated into turkeys. For instance, if material containing the histomonads but free from Heterakis is administered to birds subcutaneously, intramuscularly, intravenously or by rectal inoculation, blackhead is usually produced. These routes of infection are, however, highly artificial, and it seems most likely that under natural conditions both Histomonas meleagridis and Heterakis gallinae are causally involved.

It may occur to some one that if blackhead is to be considered a complex infection on the grounds I have just outlined, then malaria, yellow fever and other diseases with insect intermediate hosts should be classified in the same way. It is clear as to these insect-transmitted diseases that no infection with the specific virus or parasite would take place were it not for the insect intermediary which carries the agent and prepares a portal of entry for it in much the same fashion as Heterakis gallinae effects an entrance for the histomonad of blackhead. To me the difference lies in the fact that the insect intermediate hosts are free-living forms, do not themselves invade the host's body and thus may not, broadly speaking, be classed as infectious agents. If hard pressed by argument I might admit that blackhead may be a borderline example of a complex infection and less definite than some of the other diseases which I wish to discuss.

RUGOSE MOSAIC OF POTATO

The situation regarding the exact etiologic explanation of the various virus diseases affecting the potato plant is admittedly difficult and confusing. This confusion, however, is not so much concerned with facts as with nomenclature, and it seems established that at least several of the diseases of potato plants are complex virus infections. Only one of these, rugose mosaic, will be discussed. This disease is one of the more serious of those affecting American potato plants. Plants with rugose mosaic frequently die before the production of tubers, or their tubers are reduced in size. The plants themselves are dwarfed and curled with rugose, abnormally hairy leaves. The lower

leaves generally have black necrotic veins, while the upper ones are mottled with light green spots.

A thorough understanding of rugose mosaic was initiated when Johnson [5] demonstrated the presence of a virus in apparently healthy potatoes of most of the standard American varieties. This virus, seemingly completely innocuous for potato plants, induced either mottling or ringspot lesions when transferred to the leaves of tobacco plants. It was present, and readily demonstrable by the inoculation of tobacco plants, in both the foliage and tubers of the potato plants. Johnson considered two possible explanations for his observations: either the normal (or possibly abnormal) protoplasm of potatoes contained a substance capable of initiating a physiologic disturbance of tobacco and tomato plants which was of an infectious nature, or potatoes were almost universally infected with a virus. Subsequent work indicated that the second possibility was the correct one. The virus has been variously called potato virus X, mottle virus, healthy potato virus or latent potato virus.

Following the demonstration of the presence of X virus in most or all healthy potato plants, workers in three different laboratories discovered quite independently that this virus played a causal role in rugose mosaic.[6] But associated with it in this disease was another virus, variously named potato virus Y, streak virus or vein-banding virus. Valleau and Johnson [6a] in their experiments separated the viruses causing rugose mosaic, through the medium of nitrogen-starved tobacco plants. Smith,[6c] on the other hand, broke up the virus complex by using what he termed plant indicators and plant filters, by taking advantage of selective insect transmission and by utilizing the unequal rates of movement of the constituent viruses in the infected hosts. Koch [6b,d] utilized mainly selective insect transmission in his analytic experiments, and his data will be largely drawn on for the present discussion.

Prior to knowledge of the universal presence of potato X virus in healthy potato plants, Schultz and Folsom [7] obtained apparent transmission of rugose mosaic from potato plant to potato plant by aphids. Koch readily confirmed this observation, using the aphids Myzus persicae and Macrosiphum solanifolii.

It was further known that when material from potato plants infected with rugose mosaic was transferred by inoculation to tobacco

5. Johnson, J.: Transmission of Viruses from Apparently Healthy Potatoes, Research Bulletin 63, University of Wisconsin, Agricultural Experiment Station, 1925.

6. (*a*) Valleau, W. D., and Johnson, E. M.: The Relation of Some Tobacco Viruses to Potato Degeneration, Bulletin 309, University of Kentucky, Agricultural Experiment Station, 1930. (*b*) Koch, K.: Science **73**:615, 1931. (*c*) Smith, K. M.: Proc. Roy. Soc., London, s.B **109**:215, 1931. (*d*) Koch, K.: Phytopathology **23**:319, 1933.

7. Schultz, E. S., and Folsom, D.: J. Agric. Research **25**:43, 1923.

plants symptoms of spot necrosis appeared on the tobacco leaves. When, however, aphids were used in attempting to transmit rugose mosaic from the potato to the tobacco plant, the lesions produced on the tobacco plants bore no resemblance to the spot necrosis caused by mechanical inoculation. Instead, the tobacco plant showed faint symptoms of quite another type—mainly, clearing along the leaf veins and general flattening of the plant. This mild disease transmitted to tobacco plants by aphids that had fed on potato plants infected with rugose mosaic proved readily transmissible in series in tobacco plants, and the agent responsible for it was shown to be a virus. Because of the character of the disease which this virus caused in tobacco plants it was called, by Valleau and Johnson, vein-banding virus, a name also adopted by Koch.

The discrepancy between the types of disease produced in tobacco plants by mechanical inoculation and aphid transmission suggested that two distinct viruses might be associated with the potato disease. From the experiments cited it appeared likely that both of these possible viruses were readily transmissible by artificial methods of inoculation, while only one of them, namely, the vein-banding virus, was transmissible by aphids. The nature of the other virus involved was suggested by the observation, mentioned earlier, that rugose mosaic was readily transmissible from potato plant to potato plant by aphids. Since these insects could apparently transmit the complete disease from potato plant to potato plant but only the vein-banding virus from potato plant to tobacco plant it seemed obvious that they were transmitting only vein-banding virus in both cases and that the hypothetic second virus must be one normally present in the potato plant. Knowledge of the ubiquity of Johnson's mottle, or potato X, virus in healthy potato plants suggested it as the second virus in the complex, and in a series of subsequent experiments the correctness of this suggestion was proved. When pure samples of mottle and of vein-banding virus were mixed and transferred to tobacco plants, symptoms of spot necrosis like that caused by rugose mosaic material resulted, though the individual viruses, inoculated separately, produced respectively only symptoms of mottle or of vein banding. Furthermore, apparently healthy potato plants known to be carriers of virus X showed rugose mosaic after inoculation with vein-banding virus alone. The experiment crucial to proof was done with seedling potatoes that were demonstrably free from virus X. In this experiment one group of seedling potatoes were inoculated with vein-banding virus alone, one group with a mixture of vein-banding and potato X virus and a third group with rugose mosaic material. The first group, receiving vein-banding virus alone, remained free from rugose mosaic, though a slight rugosity and mottling of the upper leaves occurred. The second and third groups, inoculated respectively with a mixture of vein-banding and X virus and with rugose

mosaic material, presented symptoms of rugose mosaic. Such experiments clearly demonstrated the complexity of the etiologic background in rugose mosaic of potato.

No explanation of the mechanism by which the two viruses supplement one another in rugose mosaic has been advanced, but it seems to be generally assumed that their destructive effect on the plant is an additive one. With the X virus as widely distributed in potato plants as it is in this country, only the vein-banding virus need be transmitted from plant to plant during an epiphytotic. Thus this particular disease, though it is in fact a complex infection, appears from an epidemiologic standpoint to be a simple infection, and, were it not for the universal presence of the symptomless infection with X virus, the changes produced would be so mild as to attract little attention. Rugose mosaic of potato serves as an admirable example of a severe and serious disease caused by the concerted activity of two mild infectious agents.

It is interesting to speculate on the state of knowledge had it happened that the potato X virus was strictly species specific and infectious only for the potato plant. Its presence would probably never have been detected, and rugose mosaic would have been considered, on all experimental grounds that could be applied, as a simple infection caused by the vein-banding virus alone.

STREAK OF TOMATO

The tomato plant has a number of virus diseases, both simple and complex, and I should like to discuss briefly one of the complex type which is known as streak. This is one of the more serious diseases of the tomato plant, though fortunately it is not extremely prevalent. The characteristic symptoms are necrotic lesions on stem, leaves and fruit. The lesions on the stem take the form of dark longitudinal streaks, and the stem itself is brittle and easily broken. The leaves show necrotic spots and patches which gradually enlarge, shriveling the leaves. On affected fruits rounded or irregular sunken blotches may occur. Young plants are usually killed by the disease.

Suspicion that tomato streak might be a complex infection seems to have been first aroused by the work of Johnson [5] with the X virus of the healthy potato plant. During the course of that work he had occasion to inoculate tomato plants with a mixture of his potato X virus and tobacco mosaic virus. The resulting disease was much more severe than that caused by either virus alone and sometimes killed the plants. The following year Vanderpool [8] made the significant observation that if streak material was dried from two to nine months and then inoculated into tomato plants it no longer produced streak but only symptoms of

8. Vanderpool, T. C.: Phytopathology **16**:311, 1926.

mosaic disease. He thus demonstrated that a strain of tobacco mosaic virus had been present in his original streak material but that the prolonged drying had destroyed some other factor essential to the production of streak. Acting on the suggestion furnished by Johnson's experiments, Vanderpool explored the possibility that the agent destroyed by prolonged drying of his streak material had been a potato virus. He inoculated tomato plants with a mixture of tobacco mosaic virus and potato mosaic virus and obtained what appeared to be typical streak. Stover [9] showed that tomato streak was produced in plants inoculated with mixtures of tobacco mosaic virus and material from any one of a number of potato diseases as well as with juice from apparently healthy potato plants, while Blood [10] induced streak in tomatoes by inoculating with mixtures of tobacco mosaic virus and juice from healthy potato plants. In view of the ubiquity of the healthy potato virus it seems entirely probable, as Valleau and Johnson [11a] pointed out, that the potato plants used in all of the experiments cited contained the healthy potato virus.

In analyzing the etiologic data in two naturally occurring epiphytotics of tomato streak, Valleau and Johnson [11b] and Jones [12] found that each was caused by the combined activity of healthy potato virus and tobacco mosaic virus. It seems established, then, that tomato streak is a complex infection in which the causative agents are X virus of potato and mosaic virus of tobacco. Either virus alone produces rather mild symptoms in the tomato plant; the combination causes a severe and frequently fatal disease, the symptoms of which are distinctive. The effect of the two viruses, as in the case of rugose mosaic of potato, is supposedly an additive one. The disease serves as an example of a complex infection in which both causative agents are normally parasitic on hosts other than the one they attack concertedly.

SWINE INFLUENZA

Swine influenza may be defined as a highly contagious acute respiratory disease caused by H. influenzae suis and the virus of swine influenza.

The clinical and pathologic features of the disease will be summarized briefly. Its onset is sudden and the morbidity in an affected herd high: practically all animals under 1 year of age become sick.

9. Stover, W. G.: Phytopathology **18**:154, 1928.

10. Blood, H. L.: Phytopathology **18**:311, 1928.

11. Valleau, W. D., and Johnson, E. M.: (*a*) Phytopathology **20**:831, 1930; (*b*) **21**:1087, 1931.

12. Jones, L. K.: Phytopathology **22**:999, 1932.

Fever, anorexia, prostration of an extreme type, cough, leukopenia and rapid respiration of a peculiar abdominal type are salient features.

The period of illness is short, varying from two to six days, and if the disease is uncomplicated recovery is almost as sudden as the onset. The mortality usually ranges from 1 to 4 per cent. Autopsy on from the third to the fifth day of illness reveals a mucopurulent bronchitis and bronchiolitis and an atelectatic pneumonia variable in both amount and distribution but not infrequently involving portions of as many as five of the seven lobes. The pneumonic areas are purplish red, depressed, firm and leathery, while the adjoining lung tissue is emphysematous, exaggerating the depressed appearance of the involved portions. In fatal cases there is, in addition, diffuse bloody pulmonary edema.

The late Dr. Paul Lewis and I began our work on swine influenza during the epizootic which occurred in the autumn of 1928. We obtained from Iowa two strains of infectious material in the form of diseased lung and bronchial exudate and both of these strains proved readily transmissible to experimental swine by nasal inoculation.[13] The experimental disease faithfully reproduced that seen naturally in the field, and was maintained in swine by serial nasal passage at three or four day intervals or by contact in the pen. At autopsy the respiratory tracts of these experimental swine were studied bacteriologically in the hope that we might learn the cause of the disease. This bacteriologic investigation reached an exciting stage promptly, for from the first passage swine inoculated with each of our strains of swine influenza material there was isolated, in pure culture, an organism similar if not identical to Pfeiffer's Haemophilus influenzae. The same bacterium was isolated thereafter from all swine infected in later passages with either strain of the disease, provided they came to autopsy within seven days after the onset of fever. Frequently no organism other than this influenza bacillus–like bacterium could be recovered from the lungs or the bronchial exudate of infected animals. Because of the similarity of our bacterium to Pfeiffer's bacillus and because of its constant association with swine influenza we called it Haemophilus influenzae suis.[14] Prejudiced perhaps, at the time, by our inclination to view Pfeiffer's bacillus as the most likely claimant for consideration as the cause of human influenza, we felt quite convinced from our findings with H. influenzae suis that this bacterium was the cause of swine influenza. In the numerous cases in which it was the only organism that could be isolated there was no choice but to consider it of etiologic importance, unless we wished entirely to deny it a role in the disease.

13. Shope, R. E.: J. Exper. Med. **54**:349, 1931.

14. Lewis, P. A., and Shope, R. E.: J. Exper. Med. **54**:361, 1931.

It was, of course, obvious that if H. influenzae suis was actually the cause of swine influenza it should fulfil Koch's postulates. The first pig inoculated intranasally with what we believed to be a pure culture became ill. The lesions produced were similar to those of swine influenza, and the organism was recovered in pure culture from the respiratory tract. This apparently positive result of our experiment with H. influenzae suis, coming when it did, was unfortunate, because it made us more certain than ever that we were dealing with the causative agent of swine influenza and quite effectively closed our minds for the time to other possibilities. The experiment was, of course, repeated in a second pig, but no illness resulted and at autopsy the animal was normal. Four other pigs inoculated intranasally with pure cultures likewise remained normal, and we began to wonder a little about our positive result. Even now, there is no certain explanation of that first experiment, provided indeed the animal had influenza as was believed at the time. We considered the possibility that H. influenzae suis might be one of those organisms that lose virulence rapidly when maintained on artificial culture mediums and spent the remainder of our first year trying to discover the cause of its loss of this hypothetic pathogenicity. All of these experiments gave negative results, which only supported our growing suspicion that H. influenzae suis was not the cause of swine influenza.

The following year four fresh epizootic strains of swine influenza were obtained from Iowa and transmitted to our experimental swine, and again H. influenzae suis was regularly encountered in animals ill of the experimental disease. In addition, the organism was isolated from 6 animals affected with the disease in the field—all that were studied bacteriologically. None of these newly isolated strains, however, possessed even the slightest pathogenicity for swine. In 1930 two new strains of swine influenza were obtained in Iowa. These proved readily transmissible, and again H. influenzae suis was the predominant or only organism that could be cultivated, but all efforts to produce the disease with the new cultures were unsuccessful. We were by now ready to abandon H. influenzae suis as the cause of swine influenza.

During the first year's work a few attempts to infect swine with sterile Berkefeld filtrates of known infectious material had been made. No illness remotely resembling swine influenza had resulted, and the results were considered negative. By 1930, when H. influenzae suis had failed so completely to fulfil the requirements of an etiologic agent, we were again ready to consider the question of a virus as the causative factor.

Swine were inoculated intranasally with Berkefeld V or N filtrates of lung and bronchial exudate suspensions, known to be infectious and

autopsies were made in four or five days. Of 10 experiments, 3 were interpreted as giving negative results, while in the remaining 7 some evidence was obtained that the injected filtrate had contained an infectious agent. The illness induced by this filtrable agent was definitely not swine influenza and—for want of a better name—was designated filtrate disease.[15] Subsequent investigation has shown that the filtrable agent possesses all the properties requisite for classification as a virus.

Clinically the filtrate disease is much milder than swine influenza, and sometimes it is so ill defined that infections are difficult to recognize. In most cases there is no elevation in temperature, while in a few a fever for one day is observed. A moderate and transient apathy and some diminution in appetite are the usual symptoms shown. The extreme prostration and the various signs of extensive involvement of the respiratory tract so common in swine influenza are not seen.

At autopsy the lesions are slight as compared with the four and five lobe pneumonia of swine influenza. The lungs show only a scant, scattered, patchy lobular atelectasis, involving as a rule not more than small portions of one or two lobes.

After the establishment of the presence of a filtrable virus in swine influenza, the situation as to the cause of the disease itself became even more confused than it had been when H. influenzae suis was suspected. Here, instead of one agent that could be looked on as of possible etiologic importance, were two such agents. The bacterium could not be completely ignored, for while it had proved apparently perfectly harmless for swine, its constant presence in so many samples of infectious material from the field and its persistence on serial passage through experimental swine kept attention focused on it. Neither could the filtrable virus be accepted as the cause of the disease without reservation, because, while it unquestionably possessed pathogenic properties for swine, the mild illness that it caused was certainly not swine influenza. Considered in the light of the current conceptions of Koch's postulate that an infectious disease is caused by a single agent, it appeared that we had reached a point in our experiments where one too many were under suspicion. It seems obvious now that our data should at once have suggested that we were dealing with a complex infection. However, at the time that we were facing the incongruity of our results, the complex natures of the two plant diseases discussed earlier were not yet fully established, so that it was a little revolutionary even to consider the idea that two infectious agents might be required to cause a single infectious disease. But unlikely as the possibility seemed it was tested by inoculating a pig intranasally with a mixture of H. influenzae suis and the virus. The animal came down with swine influ-

15. Shope, R. E.: J. Exper. Med. **54**:373, 1931.

enza. With this lead a number of further experiments were carried out, and in these the effect of the virus alone and of the bacterium alone were carefully controlled. The results in all were the same: swine receiving H. influenze suis alone remained normal clinically and were normal at autopsy; swine receiving virus alone acquired only the mild filtrate disease; those receiving mixtures of H. influenzae suis and the virus presented characteristic swine influenza. From these experiments it was evident that swine influenza is a complex infection caused by the concerted action of H. influenzae suis and swine influenza virus.[15]

The cooperative mechanism by which the two agents act in causing swine influenza is not definitely known. It seems likely, though, in view of the apparent complete harmlessness of the bacterium alone, that the virus, in damaging the respiratory epithelium as it does, creates both a portal of entry and a favorable medium for the bacterium. Endowed with the invasive assistance of the virus, H. influenzae suis probably behaves in the swine respiratory tract in much the same fashion as might another bacterium possessing invasive properties by its own right. That the assistance is not entirely one sided, however, is indicated by consideration of the histology of the disease. Lesions clearly bearing the imprint of virus activity are much more extensive in swine influenza than they are in filtrate disease, indicating that the pathogenicity of the virus is enhanced by the concomitant presence of the bacterium. From this it appears probable that swine influenza represents a synergistic complex infection in which each agent enhances in some way the pathogenicity of the other.

TYPE III CORYZA OF FOWL

Under natural conditions the domestic fowl is subject to an uncomplicated coryza, not unlike the common cold of man, in which the inflammatory reaction is limited to the mucosa of the nasal passages and the communicating portions of the orbital tract. A unilateral or bilateral mucopurulent nasal discharge is the salient observable feature. Nelson [16] classified the disease into three types on the basis of length of incubation period and duration of coryzal signs. Type I has a short incubation period of from one to three days and a short duration of two weeks or less. Type II has a long incubation period of from nine to thirty days and a long duration of two months or more. Type III has a short incubation period, like type I, and a long duration, like type II. Early in his work Nelson suggested tentatively that coryza III was the basic form of the disease and that coryzas I and II were variants which tended to revert to it with continued passage through susceptible fowl. This suggestion was not entirely borne out when the causative

16. Nelson, J. B.: J. Exper. Med. **58**:297, 1933.

agents of each type were finally determined. While only coryza III has proved to be a complex infection, it is necessary for clarity that the other two types be brought into the present discussion.

The causative agent of coryza I was the first to be discovered. It proved to be a hemophilic bacillus of very fastidious growth requirements [17] and was named Haemophilus gallinarum. In pure cultures it reproduced quite faithfully the characteristic features of coryza I in fowl. H. gallinarum was also regularly present in the exudate from fowl with type III coryza, but pure cultures which had been isolated and transferred a few times on artificial mediums before testing in fowl failed to reproduce the type III disease. Instead they caused only coryza like type I. The relation of H. gallinarum to coryza of type III, with which it was always associated, was thus not very clearly defined. However, it was known that birds which had recovered from infection with H. gallinarum acquired coryza of slow onset and long duration when inoculated with exudate from fowl which had coryza III, and that H. gallinarum did not become established in their nasal passages. This modified coryza III in haemophilus-immune birds thus resembled coryza II both clinically and with respect to the uniform absence of H. gallinarum. Subsequently Nelson found that coryza II was caused by a minute gram-negative unclassified agent that he has termed the coccobacilliform bodies.[18] The discovery of the cause of coryza II immediately suggested the possibility that coryza III might be a complex infection and have as its causative factors the agents separately responsible for coryzas I and II, namely, H. gallinarum and the coccobacilliform bodies. The first actual demonstration of the presence of the coccobacilliform bodies in coryza III was effected in a group of 5 birds that had recovered from infection with H. gallinarum and subsequently were inoculated with coryza III exudate.[19] In these birds, after a long incubation period coryza like type II developed, and coccobacilliform bodies were demonstrated in all. From one of the birds the bodies were isolated in pure form in tissue culture. This strain, though deriving originally from coryza III material, was essentially the same as strains isolated from coryza II material.

It was now established that exudate from birds infected with coryza III contained both H. gallinarum and the coccobacilliform bodies. It could be demonstrated, moreover, that both agents were present throughout the entire course of the disease. This observation was contrary to the accustomed behavior of the two agents when injected in pure culture. The coccobacilliform bodies had seldom been demon-

17. Nelson, J. B.: J. Exper. Med. **58**:289, 1933; footnote 16.
18. Nelson, J. B.: J. Exper. Med. **63**:515, 1936; **64**:749 and 759, 1936.
19. Nelson, J. B.: J. Exper. Med. **67**:847, 1938.

strable before the tenth day, while H. gallinarum had seldom maintained an existence in the host for longer than two weeks.

The question of a complex etiologic background of type III coryza was finally settled by inoculating birds with mixtures of pure cultures of H. gallinarum and the coccobacilliform bodies. In these experiments the infectivity of each component alone was also controlled. In all the birds inoculated with the mixtures a type III coryza developed, of short incubation period and long duration, and both agents were regularly demonstrable throughout the course of the disease. In birds infected with the two components separately coryza I developed when they had received H. gallinarum, and coryza II when they had been given the coccobacilliform bodies. It is clear from the experiments outlined that the combined action of the two infective agents adequately accounts for the cause of type III coryza.

Nelson is of the opinion that H. gallinarum and the coccobacilliform bodies when present together in the nasal passages of the host cooperate in producing an effect that neither is able to accomplish alone. It is clearly a synergistic or, in the older sense (since both agents benefit by the association), a symbiotic reaction. The rapidly multiplying H. gallinarum creates a favorable environment for the immediate development of the coccobacilliform bodies. The latter, evidently by reason of their tendency to persist in the host, prolong the residence of H. gallinarum.[19]

TULIP BREAKING

Tulip breaking is one of the rare diseases in which the infected host is improved from a commercial and esthetic point of view. The chief symptom develops in the flowers: these, instead of being the usual solid color, become beautifully variegated, striped and mottled. This change in the flower's color is due to segregation of the anthocyanin pigment in the epidermis of the petals as fine featherings about the margin or in irregular stripes up the middle of each segment. Between these brightly colored streaks appear patches of more or less clear ground color, usually yellow or white. In addition to the flower symptoms, some tulip varieties show striping or mottling of the leaves.

Tulip breaking has been quite generally considered, in recent times at least, to be caused by a virus, and it has long been known to be readily transmissible by inoculation. Certain aspects of the disease, however, suggested to McWhorter that it was not a simple infection. For instance, in natural spread tests great variation in the types of breaks occurring were noted: the flowers of some infected plants were extremely dark, while those of other infected plants were very light. These two types of disease manifestation tended to be maintained in subsequent serial passage to normal tulips. Then, too, in some typically

"broken" clumps of tulips, distinctly darker or lighter flowers might appear at the margins of the clumps. This observation suggested to McWhorter that the light and dark "breaks" might be accounted for by two viruses which move at unequal rates through clumps and hence induce different symptomatic expressions in different shoots of the same clump. He therefore advanced the theory that tulip breaking, as it occurs naturally, results from the action of two viruses.[20] One of these viruses carries a color-adding factor and produces no visible effect on leaves; the other removes flower color and strongly stripes the leaves.

In subsequent work McWhorter proved the correctness of his theory by segregating each of the viruses, by studying their separate activities in tulips and by demonstrating that known mixtures of the two produced typical breaks.[21] The two viruses in pure form cause singularly distinct symptoms. The color-removing virus (tulip virus I) inhibits chlorophyll formation, greatly restricts growth and is directly responsible for the recognition of tulip breaking as a disease. It is an extremely lethal virus, and in McWhorter's experience all plants infected with it in pure form died out during the season in which they exhibited symptoms. The color-adding virus (tulip virus II), on the other hand, has no effect on the ground tissue of the flower or on the ground color, stimulates epidermal pigmentation, has no visible effect on the leaves and has little effect on growth. It is not a lethal virus. Mixtures of the two viruses in the proper proportions reproduce faithfully the true picture of tulip breaking. The correct proportions are, however, definite within quite narrow limits. If the proportions found necessary by McWhorter are true criteria, a typical break may be considered an expression of viruses I and II in which the concentration of the color-adding II is at least ten times that of the color-removing I. Mixtures in these proportions appear to be physiologically balanced, and virus II prevents the lethal and growth-inhibiting effect of virus I. Because of this inherent antagonism between the two naturally associated viruses, McWhorter has applied the term "antithetic" to describe their mutual relationship in the production of tulip breaking. Tulip breaking thus differs from the other complex infections already discussed, in which the end results were achieved by additive effects of the two agents.

INFECTIOUS MYXOMATOSIS OF RABBITS

Infectious myxomatosis may be defined as an acute, highly infectious, very fatal disease of rabbits caused by Virus myxomatosum. Its salient observable features are hyperemic, edematous, myxomatous swellings at the site of inoculation followed by similar swellings at all

20. McWhorter, F. P.: Phytopathology **22**:998, 1932.
21. McWhorter, F. P.: Phytopathology **25**:254, 1938.

mucocutaneous junctions. Death ensues as a rule in from eight to twelve days after inoculation. Recoveries are almost unknown.

Many will doubtless consider that infectious myxomatosis has little claim to inclusion in a discussion of complex infections. However, the disease bears certain similarities to known members of that group, and I am including it in order to point out these resemblances.

Several years ago infectious fibroma was observed occurring naturally in wild cottontail rabbits. The causative virus proved readily transmissible to laboratory rabbits and in them produced a local swelling at the site of inoculation, comprised largely of young connective tissue cells and resembling the growths occurring naturally in the cottontail rabbits.[22] The disease was named infectious fibroma, and the causative agent became known as the fibroma virus. In domestic as well as in cottontail rabbits the disease was entirely benign. There were no general signs of illness; the sole effect of the virus was the production of a fibromatous swelling at the site of inoculation; and the disease was not contagious. In all of these respects infectious fibroma differed markedly from infectious myxomatosis, which was highly fatal, highly contagious and characterized by occurrence of metastatic lesions at all mucocutaneous junctions. The fibroma did, however, bear a superficial resemblance to the local lesions produced by myxoma virus in that young connective tissue cells formed the bulk of both growths. Because of this superficial similarity, the possibility of an immunologic relationship between the causative agents of the two diseases was considered.

Domestic rabbits that had recovered from fibroma were inoculated with myxoma virus. In some of these only a localized myxomatous swelling developed at the site of inoculation, and there were none of the general symptoms of myxomatosis. In others a disease developed that clinically looked like true myxomatosis but differed in that the animals recovered. A very few of the rabbits which had recovered from fibroma died of myxomatosis.[23] It was clear from such results that previous infection with fibroma virus usually made rabbits quite highly resistant to myxoma virus. That this resistance was not of the same category as the cross immunity produced by two identical viruses for one another was indicated by the fact that rabbits which had recovered from fibroma almost always acquired lesions and signs of myxomatosis; they were incapable of destroying injected myxoma virus, and their serum did not neutralize myxoma virus sufficiently to prevent death, though Berry and Lichty[24] subsequently showed that the serum

22. Shope, R. E.: J. Exper. Med. **56**:793, 1932.
23. Shope, R. E.: J. Exper. Med. **56**:803, 1932.
24. Berry, G. P., and Lichty, J. A., Jr.: J. Bact. **31**:49, 1936.

of such rabbits does prevent the appearance of local myxomatous lesions. The relationship in the reverse direction was that of a true cross immunity, for rabbits which had recovered from myxoma were solidly immune to fibroma; they inactivated injected fibroma virus, and their serum neutralized fibroma virus. The conclusion was drawn that, though the fibroma and myxoma viruses were immunologically related, they were not identical viruses and fibroma virus did not constitute merely a mild strain of the myxoma agent.[23]

Rather it seemed that the differences could be explained best on the basis of partial duplication of the antigenic components comprising the two viruses, the myxoma virus containing all the components found in the fibroma virus but the fibroma virus being antigenically only a partial replica of the myxoma virus. In line with this explanation the possibility was considered that the agent so long known as Virus myxomatosum might be, as Rivers [25] had, on other grounds, previously suggested, actually composed of more than one virus. The immunologic relationships between the fibroma and myxoma viruses were in accord with the possibility that Virus myxomatosum might be composed of fibroma virus and some other perhaps hitherto unknown virus. Such an explanation would adequately explain why Virus myxomatosum immunized completely against fibroma virus, one of its components, while the fibroma virus, being but a part of Virus myxomatosum, gave correspondingly only partial immunization.

In an effort to demonstrate this hypothetic complexity in Virus myxomatosum, serial passage of the virus through hosts that conceivably might favor survival of one or the other component was conducted. It was reasoned that, since fibroma virus had come originally from wild cottontail rabbits and was apparently a natural pathogen for this species, it might be segregated and obtained pure by the serial passage of Virus myxomatosum through cottontail rabbits. In like manner, it seemed possible that serial passage of Virus myxomatosum through fibroma-immune rabbits might result in the survival of only the hypothetic second virus. However, both sets of experiments yielded only unaltered Virus myxomatosum,[26] and no experimental evidence was obtained to support the suspicion that Virus myxomatosum might be a virus complex.

But Berry and Dedrick [27] were more successful in demonstrating the complexity of Virus myxomatosum, though they visualized this complexity in a light quite other than that outlined in the preceding paragraph. They considered the possibility that the fibroma and

25. Rivers, T. M.: Proc. Soc. Exper. Biol. & Med. **24**:435, 1926-1927.
26. Shope, R. E.: J. Exper. Med. **63**:33 and 43, 1936.
27. Berry, G. P., and Dedrick, H. M.: J. Bact. **31**:50, 1936.

myxoma viruses might be but different strains of one basic virus and, if this were the case, might be amenable to transformation in a manner similar to that employed by Griffith [28] with pneumococcic types.

The method they employed consisted in inoculating domestic rabbits with a mixture of active fibroma virus and heat-inactivated myxoma virus. In animals inoculated with such mixtures myxomatosis developed that was typical in all respects. This result, startling as it seems, can be duplicated readily, and neither Hurst [29] nor I have experienced any difficulty in repeating the experiments. In performing a transformation experiment the myxoma virus may be heated in sealed tubes for thirty minutes at any temperature between 60 and 75 C. Even the lowest temperature used is several degrees above that required to destroy the infectivity of myxoma virus. The heat-inactivated myxoma virus is then mixed with a small amount of active fibroma virus before injection into test rabbits. Typical myxoma virus, readily transmissible in series and with all its killing propensities intact, is recoverable from the inoculated rabbits. The disease that this transformed virus causes bears no more resemblance to infectious fibroma than did the original myxomatosis from which the virus for heating was derived.

The experiments of Berry and Dedrick have been extremely painstakingly controlled, and it seems impossible that the result is explainable on the basis of survival of traces of active myxoma virus in their heated material. Furthermore, it appears unlikely that the thermostabile component of myxoma virus is itself a virus as one usually thinks of such an agent, because alone it produces no signs of infection in rabbits, it confers not even partial immunity to myxomatosis, and it does not, of itself, multiply in the rabbit. Berry and Dedrick have not yet expressed their own views as to the nature of the transforming agent, though in a preliminary publication they have suggested that it may lend virulence to fibroma virus in a manner analogous to bacterial haptens. Whatever its nature, however, it seems established that the Berry-Dedrick factor, in conjunction with the benign fibroma virus, causes a disease that is unsurpassed among infectious maladies for pathogenicity and deadliness. The factor may yet prove to be viral in nature, peculiar only with regard to its high thermal inactivation point and its requirement of the concomitant presence of fibroma virus for multiplication within the rabbit host; and it does multiply, because, once established, it is indefinitely transmissible in series.

This completes my consideration of the complex infections, and in concluding I should like to emphasize two of the features of their causative agents that were not stressed earlier. The first concerns the

28. Griffith, F.: J. Hyg. **27**:113, 1928.
29. Hurst, E. W.: Brit. J. Exper. Path. **18**:23, 1937.

frequent extreme mildness of one or both of the agents when acting individually. In rugose mosaic of potato, for instance, an infection of potatoes with either the mottle or the vein-banding virus alone would attract little or no attention; and in swine influenza the bacterial component alone causes no recognizable illness, while the virus alone produces only an extremely mild disease. The combined effect, in all cases except tulip breaking, far exceeds that to be expected from the known activities of the agents singly. The second feature to be stressed concerns the occasional high pathogenicity of one of the components of an etiologic complex for another host. In tomato streak, for instance, the tobacco mosaic virus component alone may cause serious disease in tobacco; while in swine influenza the virus component alone will produce serious and sometimes fatal pneumonia in ferrets and regularly fatal pneumonia in white mice. If one wished to expand on the possible implications of these two features of the agents involved in the complex infections, one could readily speculate that they might have an important bearing with regard to the future appearance of new diseases of either simple or complex etiologic background. For instance, there is no way of knowing what a congregation of latent and, alone, impotent agents may be lurking about, awaiting only the addition of another mild agent to cause serious disease. Neither can it be foretold when one of the mild agents of a complex will forsake its partner and take up residence alone in a host for which it is highly pathogenic. But these possibilities are purely speculative.

Another fact of considerable interest that was not emphasized is that in the case of each one of the seven diseases discussed a simple "one agent" causation was in the beginning considered probable or proved. Some minor discrepancy or fortuitous observation led eventually to the discovery that two agents were involved. This seems to indicate that those who are studying infectious diseases are thinking largely in terms of one instead of two causal agents. They are still under the influence of the spirit of Koch's postulates and find it difficult to abandon even occasionally the concept that for each infectious disease there must be a single specific etiologic agent. If there is anything at all to be learned from present knowledge of the complex infections, it is that an infectious agent must fully explain and account for all of the features of a disease with which it is associated before it is accepted as the sole cause of that disease. Investigators must think more often in terms of two factors if they are to gain full understanding of all the infectious diseases.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—Marcos Fernan-Nunez, professor of pathology and bacteriology in Marquette University, Milwaukee, is now chairman of the cancer committee of the State Medical Society of Wisconsin.

Julius M. Rogoff, visiting professor of physiology in the University of Chicago, has been appointed professor of endocrinology in the University of Pittsburgh.

C. H. Andrewes, pathologist, National Institute for Medical Research, and H. M. Turnbull, professor of morbid anatomy, London Hospital, have been elected fellows of the Royal Society.

William H. Park, director of the laboratories of the New York City Health Department from 1894 until his retirement in 1936, has died, aged 76 years.

William C. Thro, professor of clinical pathology at Cornell University Medical College from 1918 to 1937, has died at the age of 64 years.

Maurice N. Richter, since 1928 assistant professor of pathology in the College of Physicians and Surgeons of Columbia University, has been promoted to a professorship and appointed executive officer of the department of pathology in the New York Post-Graduate Medical School and Hospital.

Robert A. Moore, assistant professor of pathology at the Cornell University Medical College, New York, has been appointed professor of pathology at Washington University, St. Louis.

Alfred Stengel, vice president in charge of medical affairs of the University of Pennsylvania and emeritus professor of medicine, died on April 10, 1939, at the age of 70. Dr. Stengel was a member of the editorial board of the ARCHIVES OF PATHOLOGY from the beginning.

CORRECTION

In the article by Dr. L. A. Emge entitled "Sarcomatous Degeneration of Transplantable Mammary Adenofibroma of the White Rat," which appeared in the July 1938 issue (ARCH. PATH. **26**:429, 1938), the letters were inserted in figure 2 in horizontal instead of vertical order, but the legends were not changed accordingly. To make the parts of the illustration correspond with the legends, the following changes are needed: C should be B, E should be C, B should be D, and D should be E.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Disturbances of the Blood and Lymph Circulation in the Abdomen. W. D. Gatch, Surg., Gynec. & Obst. **66**:322, 1938.

The problem of whether the intra-abdominal circulation is impeded by greatly increased intra-abdominal pressure was solved experimentally by determining the systolic blood pressure, the intra-abdominal pressure and the tension in the vena cava and then elevating the intra-abdominal pressure by injecting physiologic solution of sodium chloride into the peritoneal cavity. As the pressure within the abdominal cavity increased, the pressure in the vena cava kept pace with it so that the two were always equal. When the intra-abdominal pressure equaled the systolic blood pressure, all flow through the abdominal organs ceased, and they became white and bloodless. Thus it seems that the heart forces enough blood through the capillaries to maintain an intravenous pressure equal to that within the abdomen and further that the intra-abdominal circulation cannot be stopped by any increase of intra-abdominal pressure lower than the diastolic blood pressure. When the pressure on a capillary equals the diastolic blood pressure, the flow of blood through the capillary occurs only in systole and consists of a series of brief spurts. The resulting volume of the blood flow is insufficient to maintain the normal activities of the tissues. Under the conditions of the experiment the absorption of salt solution is slow.

The effect of bowel distention and that of venous obstruction limited to a single abdominal organ were likewise studied. It was demonstrated that the venous outflow from a distended bowel decreases as the intraintestinal pressure increases and ceases when the intraintestinal pressure equals the systolic blood pressure; that distention of the bowel causes no venous congestion thereof; that the bowel is not injured by intraintestinal tension short of that necessary to rupture it, provided the pressure is applied for a short time only; that after deflation the bowel can be made to contract normally and displays no microscopic evidence of damage; that the function of the bowel as shown by its power of absorption is maintained fairly normal in the presence of intraintestinal pressure lower than the diastolic blood pressure but ceases when the intraintestinal pressure is higher than this; that distention of the bowel causes no edema of its wall but diminishes or abolishes secretion by its mucosa; that loops of bowel kept tightly distended for a number of hours gradually increase in diameter and may remain viable if the pressure within them does not increase as fast as their walls are stretched, enlargement under these conditions diminishing the pressure on the capillaries of the bowel wall and permitting a resumption of the blood flow. The effects of distention on the appendix and on the cystic ovary are much the same as on the intestine. The gallbladder, however, behaves differently. After ligation of the cystic duct the gallbladder contracts within a few hours, which is due to rapid absorption of water from its contents; ligation of the common duct alone brings about great distention of the gallbladder, for the power of removing water from its contents is insufficient to cope with the constant arrival of more fluid by way of the cystic duct.

In the presence of venous obstruction manometer readings indicate a rise of pressure equal to that of the systolic blood tension, proving that in venous obstruc-

tion the systolic blood pressure is transmitted through the capillaries into the veins. Under these circumstances the capillaries rupture, and there follows a massive extravasation of blood into the tissue spaces.

Disturbances of the blood flow are always accompanied by alterations of the lymph flow: An elevation of intraintestinal pressure beyond a certain level abolishes the flow of lymph in the abdominal wall; a slight rise in intravenous pressure increases the flow of lymph, while complete venous obstruction abolishes it.

WARREN C. HUNTER.

ALLERGIC HYPERERGIC INFLAMMATION EVOKED BY AUTOSERUM. W. EICKHOFF, Virchows Arch. f. path. Anat. **301**:264, 1938.

Attempts to sensitize an animal to its own blood or serum have led to conflicting results because, according to Eickhoff, there could be no certainty that the blood or serum used for reinjection had the same chemical composition as that used for the first injection. Eickhoff overcame this objection by withdrawing from rabbits and guinea pigs by cardiac puncture a quantity of blood that would yield sufficient serum for a first, or sensitizing, injection and a second, or provocative, one. The two portions of serum were inspissated in vacuo at the same time by the method of Flosdorf and Mudd. Sealed under vacuum, the material was kept on ice until needed. Then sufficient distilled water was added to restore the original volume of the serum. Solutions so prepared were clear and colorless. The first injection was made three weeks after the cardiac puncture, and the second three weeks after the first, both being made subcutaneously and at the same site. The first injection had no detectable local effect. The second injection of the autoserum led to local swelling of the skin. Histologic examination of such an area forty-eight hours after the injection revealed edema of the subcutis, separation of its fibrous elements and marked cellular infiltration, the picture being identical with that of Arthus' phenomenon. The infiltrating cells were leukocytes, eosinophils and histiocytes. Tissues examined at later periods showed fibroblastic proliferation. If the second dose of autoserum was given by intravenous or intracardiac injection it produced anaphylactic shock.

O. T. SCHULTZ.

Pathologic Anatomy

STENOSIS OF THE SPLENIC VEIN IN CHILDHOOD. J. HÖRA, Virchows Arch. f. path. Anat. **300**:670, 1937.

Thrombosis of the splenic vein in the adult leads to a characteristic triad of splenomegaly, gastric hemorrhage and secondary anemia, which permits clinical recognition of the condition. In children a somewhat similar complex of symptoms occurs, but operation or necropsy fails to reveal thrombosis or obstruction of the splenic vein. The noncommittal clinical term "stenosis of the splenic vein" has been applied to the condition in children. Failure to detect obstruction of the splenic vein has led to the supposition that the obstruction to the splenic circulation lies within the spleen itself. In children splenectomy usually leads to recovery; hence there are few records of observations made at necropsy. Höra presents a detailed histologic study of 2 spleens removed surgically, the first from a boy aged 8 years and the second from a girl aged 9 years. Neither grossly nor microscopically was there evidence of disease or of obstruction of veins outside the spleen. In each instance the spleen was enlarged to about four times the normal size. The splenomegaly was due to hyperplasia of the red pulp, with marked prominence of the sinusoids. Congestive hemorrhages had occurred in the trabeculae, and siderofibrous nodules had been formed, but there were no alterations within the spleen itself that would account for interference with the

circulation through the spleen. The cause must be sought outside the spleen. In the absence of anatomic changes in the splenic vein one must consider functional stasis of the vein or obstruction of the portal circulation.

O. T. SCHULTZ.

SPECIFIC CELLULAR CHANGES DUE TO ELECTRICITY. S. JELLINEK, Virchows Arch. f. path. Anat. **301**:28, 1938.

As characteristic and specific effects of the passage of electricity through the body, the director of the Institute for Electropathology of the University of Vienna describes two types of cellular change. These occur in and immediately adjacent to electric marks that reveal no evidence of burning. The first is a spiral deformation of the nuclei of the media of blood vessels, as if the nuclei had been subjected to a twisting force in opposite directions applied to their poles. Such deformed nuclei were observed in the tissue adjacent to scars of lesions caused by electric current and sustained, respectively, three and five years previous to death, an observation which the author characterizes as remarkable. The other type of change was noted especially in the epithelium of the hair follicles. The nuclei were elongated, needle shaped, and had a wavelike geometric arrangement, which the author likens to the lines of force in an electric field. Both types of change were observed in rabbit tissues experimentally excised by the high frequency electric surgical knife, at a slight distance from the surface in contact with the knife. These observations, the author suggests, may be indicative of possible danger in the use of the high frequency knife or of the diathermy current.

O. T. SCHULTZ.

RELATION OF ANOMALIES OF THE CIRCLE OF WILLIS TO ANEURYSM OF THE BASE OF THE BRAIN. A. SLANY, Virchows Arch. f. path. Anat. **301**:62, 1938.

Brief summaries are presented of 26 cases of aneurysm of the circle of Willis that led to fatal intracranial hemorrhage. The subjects had come to necropsy in Priesel's institute, Vienna, during the preceding ten years. In 14 cases the aneurysm was associated with an anomaly of the vascular circle. The frequency of the association suggests that there may be a causal relationship between anomaly and aneurysm of the circle of Willis.

O. T. SCHULTZ.

THE PYELONEPHRITIC CONTRACTED KIDNEY. T. FAHR, Virchows Arch. f. path. Anat. **301**:140, 1938.

In a 46 page article, in which the material is excellently organized and set forth in language easy to read, Fahr presents a study of the pathologic aspects and a discussion of the genesis of the pyelonephritic contracted kidney, based on 80 cases. He traces the process from its inception as subacute pyelitis, which may involve the entire pelvis or only one or more calices, through stages of ascending proliferative interstitial inflammation of the medulla, to a final stage of almost complete replacement of the kidney by fibrous tissue, with contraction. He agrees in general with Staemmler but does not agree that in every instance the process passes through a stage of cystic dilatation of the tubules or a stage of struma-like transformation of the kidney. He thinks it is necessary to recognize two different types of ascending inflammatory process. In one of the ascending interstitial inflammation is more diffuse, spreads more rapidly upward into the kidney, with fibrous replacement of tubules and glomeruli but without struma-like transformation of the organ. In the other type the process ascends more slowly and is associated with fibrosis of the medulla; with this change the cystic stage described by Staemmler develops. But such cystic transformation is not always the result of the ascending inflammation. It may have existed previous

to the development of the latter in hypogenetic areas of the kidney or in an organ which is diffusely hypoplastic. Such hypogenetic areas seem to be more prone to ascending inflammation than previously normal kidneys. Hypogenetic renal tissue is also more prone to malignant nephrosclerosis and chronic glomerulonephritis. Fahr suggests that the interstitial nephritis associated with dwarfism or rickets is ascending pyelonephritis in a congenitally hypogenetic kidney. He believes it is necessary to recognize a form of nephritis which he terms hypogenetic nephritis. He considers chronic ascending nephritis the most frequent form of renal inflammation.

O. T. SCHULTZ.

WIDENING OF THE CRANIAL SUTURES. A. E. SITSEN, Virchows Arch. f. path. Anat. **301**:287, 1938.

Sitsen gives a detailed description of the pathologic alterations of the bone at the suture margins in 5 cases of widening of the cranial sutures. The series includes 1 case of spongioblastoma of the brain in a child, 1 case of sympathicoblastoma in a child with metastasis to the skull and 3 instances of suppurative osteomyelitis of the cranial bones. Widening of the sutures may be brought about by abnormal mobility of the irregular bone margins or by separation of these bone margins. Increased mobility may be due to (1) resorption of bone and its return to the fetal state, as brought about by increased intracranial pressure, (2) to destruction of bone by a metastatic tumor situated near a suture and (3) to destruction of bone by suppurative inflammation. Actual separation of the suture margins requires an increase in intracranial pressure and can occur only in early life, when the sutures have not yet united, or in later life, when the bony margins of the sutures are altered or destroyed.

O. T. SCHULTZ.

CARCINOGENETIC ACTIVITY, STRUCTURE AND CHEMICAL REACTIVITY OF POLYNUCLEAR AROMATIC HYDROCARBONS. L. F. FELSER, Am. J. Cancer **34**:37, 1938.

A survey and analysis are made of the chemical and biologic investigations of hydrocarbon carcinogenesis, with significant conclusions bearing on further work in this field.

VARIATION IN THE CREATINE CONTENT OF HUMAN MUSCLE AT AUTOPSY. C. R. LINEGAR, T. T. FROST and V. C. MYERS, Arch. Int. Med. **61**:430, 1938.

The creatine content of the heart is low at birth but progressively increases until within a few months it is equal to that found in adults. The saturation level for the creatine of cardiac muscle is reached much earlier than that for the creatine of voluntary muscle. In cardiac decompensation the creatine content of the heart, i. e., of both the left and the right ventricle, is definitely lowered in comparison with average values. It is also usually slightly lowered in diabetes and carcinoma. On the other hand, the creatine content of the muscles of the left and right ventricles may be considerably increased in uremia uncomplicated with heart failure and in pneumonia in some cases. The creatine content of voluntary muscle (the pectoralis major muscle being taken as an example) is reduced in diabetes and carcinoma and increased in uremia uncomplicated with heart failure and in the pneumonias, compared with average values. The creatine content of cardiac and voluntary muscle may be reduced or increased in fairly constant ratios, but the major evidence points to the conclusion that variations in these two distinctly different muscles are not related except when the creatine contents of both voluntary and cardiac muscle are elevated, probably as a result of retention of nitrogen.

FROM AUTHORS' SUMMARY.

Pathologic Chemistry and Physics

MINERALS IN NORMAL AND IN PATHOLOGIC BRAIN TISSUE, STUDIED BY MICROINCINERATION AND SPECTROSCOPY. L. ALEXANDER and A. MYERSON, Arch. Neurol. & Psychiat. **39**:131, 1938.

The microincineration method enables one to determine the relative distribution of ash in various parts of normal and of pathologic nerve tissue. The gray matter is rich in ash; the white matter is poor in ash. The mineral distribution in the various parts of the neuron varies. For instance, in the ganglion cell the nucleus and Nissl bodies contain rich deposits of ash, while the intracellular neurofibrils contain little or no mineral ash; the lipoid of the myelin is free from mineral ash, and the axons contain small amounts. With hemorrhages, inflammation, tuberous sclerosis and tumors there is hypermineralization; in ischemic necrosis, in plaques of multiple sclerosis, in areas of cortical atrophy and in cell disease of patients with amaurotic family idiocy there is demineralization.

Spectroscopic studies revealed that the white matter contains about twice as much phosphorus as the gray matter, while the gray matter is richer in sodium, calcium and magnesium. The dried substance of the gray matter is richer in iron, but potassium, manganese and copper are about evenly distributed. By the spectroscopic method the relative mineral content was studied in the gray and white substances of the brains of newborn infants and in those of normal adults, also brains showing one or the other of the following conditions: softening of the brain, multiple sclerosis, dementia paralytica, encephalitis due to poisoning with lead, cerebral edema and tumors. In multiple sclerosis the plaques showed twice as much iron as normal white matter of the same brain; calcium was decreased in the plaques, and phosphorus showed no significant changes. The reason for the discrepancy between the findings by the spectroscopic and the microincineration methods of studying multiple sclerosis was that in multiple sclerosis the scavenger glia cells appropriated, as it were, most of the minerals and thus depleted the tissues. In dementia paralytica no absolute increase of iron was found, while in lead encephalitis more lead was deposited in the gray than in the white substance.

GEORGE B. HASSIN.

SERUM ENZYMES AND FERMENTATION TESTS. N. E. GOLDSWORTHY, J. E. STILL and J. A. DUMARESQ, J. Path. & Bact. **46**:253, 1938.

Horse serum contains amylase and maltase even after storage for many weeks at 4 C. These enzymes can lead to a false interpretation when the serum is added to a fermentation medium. To avoid this difficulty, the serum should be heated for sixty minutes at 65 C. before being added to the medium. There is disagreement between the results of some authors who have investigated the enzyme content of serum in various animal species. Certain factors other than enzymes which may influence the apparent result of a fermentation test are discussed.

FROM AUTHORS' SUMMARY.

CENTRIFUGATION OF THE ELEMENTARY BODIES OF INFECTIOUS MYXOMATOSIS OF THE RABBIT. C. E. VAN ROOYEN and A. J. RHODES, Zentralbl. f. Bakt. (Abt. 1) **140**:117, 1937.

A speed of 15,000 revolutions per minute for two hours caused deposition of the elementary bodies. The supernatant fluid was noninfective, but the centrifuged sediment containing the elementary bodies, on intradermal injection into rabbits produced typical myxomatous papules.

PAUL R. CANNON.

Microbiology and Parasitology

SUPERINFECTION IN MALARIA. L. T. COGGESHALL and H. W. KUMM, J. Exper. Med. **68**:17, 1938.

Protection tests were utilized to determine the effect of superinfection on the potency of immune serum from monkeys chronically infected with Plasmodium knowlesi. The results of these tests showed that: 1. In two groups of monkeys with comparable infections the immune serum of 8 monkeys which had been superinfected on seven separate occasions over a period of two months was much more potent than that of a group of 7 monkeys in which the chronic course of infection was allowed to continue without the introduction of a superinfection. 2. After a series of nine more intense superinfections the serum from the same two groups of monkeys contained no demonstrable protective antibodies. 3. The serum from 8 of the 10 monkeys in the original two groups showed a relatively high concentration of protective antibodies following a month's rest and a single superinfection. 4. The results of the experiments indicate that it is possible to increase the potency of immune serum by superinfections but that it is also possible to obtain a decrease in the protective property of the serum by too severe superinfections.

FROM AUTHORS' SUMMARY.

CUTANEOUS INFECTIVITY IN POLIOMYELITIS. W. J. GERMAN and J. D. TRASK, J. Exper. Med. **68**:125, 1938.

Bilateral olfactory neurectomy did not prevent experimental poliomyelitis following intravenous or intracutaneous inoculation of the virus. Various operative procedures increased the susceptibility of monkeys to this infection.

FROM AUTHORS' SUMMARY.

A POSSIBLE MECHANISM OF LOWERED RESISTANCE TO PNEUMONIA. W. J. NUNGESTER and R. G. KLEPSER, J. Infect. Dis. **63**:94, 1938.

Mucin injected intrabronchially favored the production of pneumonia in rats sprayed one day later with pneumococci. Certain factors, as exposure to cold, prolonged deep ether anesthesia or alcoholic intoxication, increased the aspiration of mucous material placed in the noses of white rats. Such factors also increased the incidence of pneumonia if pneumococci and mucin had been inoculated intranasally. Cold or alcoholic intoxication were found to interfere with the closing of the glottis, thereby permitting the aspiration of mucin and pneumococci.

FROM AUTHORS' SUMMARY.

TUBERCULOUS INFECTION FROM TALCUM USED TO DRY GLOVES USED FOR NECROPSIES. R. OEHNELL, Zentralbl. f. allg. Path. u. path. Anat. **69**:324, 1938.

Dissemination of tubercle bacilli by talcum powder used to dry gloves worn at necropsies is reported. Oehnell carried out his researches designated A and B, with observations as follows. At hospital A, from 8 to 9 bodies dead of pulmonary tuberculosis were examined each month. The attendants frequently washed their rubber gloves hurriedly with soap and water, then gave them a preliminary drying on a towel. Talcum sprinkled from a shaker was used to complete the drying and often was scattered in a cloud when imprisoned air was used to turn the gloves right side out. Guinea pigs placed within a meter of the tablet on which the excess talcum fell, developed widespread tuberculous lesions. Others exposed to talcum which had not come in contact with gloves remained free from disease when kept about 10 meters from the tablet.

In hospital B, the gloves were powdered in a large glass cylinder kept in an antechamber of the morgue. Talcum was used repeatedly until the container was empty. Only from 1 to 2 bodies dead of tuberculosis were examined in this institution per month. Guinea pigs kept close to the talcum container remained healthy and had no tuberculous conditions post mortem. The studies indicate a possible source of tuberculous and other infections in man exposed to talcum dust contaminated by bacteria adherent to gloves used in morgues.

GEORGE J. RUKSTINAT.

BRUCELLIASIS IN JAPAN AND MANCHUKUO. H. HIROKI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**:382, 1938.

From 10 to 20 per cent of cattle in Japan were found infected with Brucella abortus. Br. abortus infection of man is not rare, but there are no known instances of infection of man with Brucella melitensis. Brucella infection is considerably greater in Manchukuo, in man as well as in cattle. The serum of about 10 per cent of the persons working on cattle and sheep farms gave positive agglutination or complement fixation with Br. abortus or Br. melitensis. Some of these had no clinical manifestations and no history of the disease.

I. DAVIDSOHN.

Immunology

BRUCELLA PRECIPITIN SYSTEMS. R. B. PENNELL and I. F. HUDDLESON, J. Exper. Med. **68**:73 and 83, 1938.

It has been shown that the precipitation by the endoantigens of the three species of Brucella of their homologous antibodies may be described by equations developed from the law of mass action. The endoantigens may be used in calibrating accurately brucella antisera. The nitrogen-containing constituent of the endoantigens does not always seem to be intimately connected with the ability to precipitate the specific antibodies.

Quantitative cross precipitation studies with goat antiserums show the three endoantigens of Brucella to be serologically distinguishable. Although the endoantigens of Brucella abortus and Brucella suis are very similar, they do not react identically, which permits the two organisms to be distinguished serologically. These differences in cross precipitation may be used to identify an organism of the brucella group or to determine the organism responsible for a brucella antiserum.

FROM AUTHORS' SUMMARIES.

IMMUNITY AFTER ENCEPHALITIS VIRUS VACCINATION. L. T. WEBSTER, J. Exper. Med. **68**:111, 1938.

Susceptible mice that are given subcutaneous or intraperitoneal injections of 15,000 intracerebral lethal doses of St. Louis encephalitis virus acquire immunity in from four to seven days to from 1,000 to 1,000,000 lethal doses given either intracerebrally or intranasally. This immunity persists for from four to six weeks, then decreases gradually and after from eight to twelve weeks disappears. More than 1,000 intracerebral lethal doses of virus given as a vaccine does not materially increase the amount or duration of the immunity; less than 1,000 doses gives little or no immunity. Test virus injected intracerebrally into immunized mice induces few lesions and is rapidly destroyed; instilled intranasally, it rarely reaches the olfactory lobes or brain. While immunity is maximum, circulating neutralizing antibodies are not detectable. Moreover, the immunity is not affected by endothelial cell blockade or by splenectomy. A few moments after the immunizing virus is given, it can be recovered from the blood in relatively high concentration.

After twenty-four hours, the blood no longer contains demonstrable virus nor do any organs thus far tested except the spleen. The brain and cord remain entirely normal. The spleen, however, becomes enlarged and harbors virus for as long as thirty days. FROM AUTHOR'S SUMMARY.

TOXIN PRODUCTION BY BACILLUS HISTOLYTICUS. L. E. WALBUM and G. E. REYMANN, J. Path. & Bact. **46**:315, 1938.

Under the experimental conditions described Bacillus histolyticus grows luxuriantly in ordinary broth containing 1 per cent peptone irrespective of the presence of dextrose. The production of toxin appears to be greatest in broth without addition of dextrose or in Bacillus coli–fermented broth. The peptone content of the medium has a considerable effect on the toxin production, the production increasing with increase in the concentration of peptone. The greatest production of toxin occurs in peptone broth containing pieces of meat. This medium gives a substantial and steadily increasing production of precisely those protein split products which are of such importance for the growth of the bacteria and for the production of toxin. B. histolyticus toxin has its maximal point of stability in the neighborhood of p_H 6. The optimal reaction for the albumose-digesting enzyme lies around p_H 7. FROM AUTHORS' SUMMARY.

AGGLUTINOGENS RESEMBLING M AND N FACTORS IN MONKEYS. P. DAHR and H. LINDAU, Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**:335, 1938.

One anti-M testing fluid clumped the red cells of some old world lower apes, while other anti-M serums failed to do it. The observation indicates that there are differences in the anti-M agglutinating serums. An explanation is offered for that difference: The M agglutinogen is composed of fractions, some of which are present in certain animal species. Occasional rabbits may have one or the other of these fractions in their red cells or in other tissues, a circumstance influencing the structure of antiserums produced by inoculating them with human red cells of group OM. The failure to find the M property in an animal species is of little significance if only one anti-M testing fluid has been used. The resemblance of the M agglutinogen of monkeys to that of man increases with the zoologic proximity to man. The N agglutinogen was found only rarely in monkeys. Here, again, the agglutination reaction, indicative of the presence of the N factor, was demonstrated only with some of the anti-N serums, showing that they, too, differ as do the anti-M serums. I. DAVIDSOHN.

ELIMINATION OF THE B AGGLUTINOGEN IN SALIVA. F. KAUERZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**:460, 1938.

The B agglutinogen of man consists of at least three fractions, B_1, B_2 and B_3. B_1 has thus far been found only in man and in some anthropoid apes. B_2 and B_3 are regularly present in rabbits, cats and some other animals. B_3 is found in guinea pigs, some dogs, elephants and some lower apes. Human anti-B serums differ in composition: Most of them have β_2 and β_3 agglutinins, which are directed against red cells with the corresponding agglutinogenic fractions; some serums have in addition to the aforementioned agglutinins β_1 agglutinin; none are known with only the β_3 agglutinin. The B agglutinogen which is eliminated in the saliva does not always consist of the same fractions that are present in the red cells of the same person; in 13 of 50 salivas of B persons only fraction B_1 was present; if these 13 persons had been tested with one of the common anti-B serums with only β_2 and β_3 agglutinins they would have been labeled noneliminators. Only

serum with the β_1 agglutinins in addition to the two other fractions can be used for a reliable determination of the elimination of B agglutinogen. By proper absorption of human anti-B serums with rabbit red cells, containing the B_2 and B_3 fractions, and with the red cells of the guinea pig, containing the B_3 fraction, serums can be prepared which hold isolated agglutinins β_1 or β_2.

I. DAVIDSOHN.

THE SHWARTZMAN PHENOMENON. G. ALBUS and K. FISCHER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**:472, 1938.

Rabbits were sensitized with an intraperitoneal injection of pollen extract. The presence of sensitization was established by means of the passive transfer of Prausnitz-Küstner. The sensitized animals were inoculated intracutaneously in one area with a filtrate of a culture of Bacillus coli and in another area with 0.3 cc. of a 1:10 dilution of the pollen extract. In both areas inflammatory reactions developed. Intravenous injections of 2 cc. of the filtrate of B. coli produced grossly and microscopically typical hemorrhagic lesions in 50 per cent of the animals. In no case did a hemorrhagic lesion develop only in one of the two prepared sites. In nonsensitized animals hemorrhagic lesions appeared only in the areas prepared with the bacterial filtrate. Normal rabbits were prepared locally by intracutaneous injections of B. coli filtrate. Intravenous injections of a mixture of pollen extract and serum from sensitized animals did not produce the phenomenon, but when animals were sensitized to the pollen and then given injections as described the phenomenon was produced in 25 per cent of them. The results indicate that the reaction that takes place between the pollen and the reagins is capable of preparing the skin for the Shwartzman phenomenon and further that the in vivo reaction between the injected pollen extract and the reagins in the sensitized animal can provoke the phenomenon in the prepared skin.

I. DAVIDSOHN.

Tumors

CLASMATOSIS IN THE MELANOBLAST. C. G. GRAND, Am. J. Cancer **33**:394, 1938.

The elimination of granules of melanin by the melanoblast occurs by active pinching off, or clasmatosis, of clumps of granules irregularly arranged along the length of its dendrites. Clasmatosis occurs either by fragmentation of the pseudopodium or by formation of a bud which breaks away from the side of the pseudopodium. The constancy of clasmatosis in the melanoblast and its occurrence in only one of the types of cell found in the tissue cultures of mouse melanoma argue against the phenomenon being accidental or due to an abnormal condition of the medium. The irregularity in the appearance of the granules in the macrophages is due to the ingestion by the macrophages of the fragmented débris of melanin previously eliminated by the melanoblasts. Clasmatosis, a physiologic mode of excretion first described by Ranvier, is at least one way in which the melanoblasts of the Harding and Passey mouse melanoma eliminate melanin.

FROM AUTHOR'S SUMMARY.

BASOPHIL ADENOMA OF THE PARS INTERMEDIA OF THE HYPOPHYSIS. A. T. RASMUSSEN and A. A. NELSON, Am. J. Path. **14**:297, 1938.

Two cases in which basophil adenoma originated from the pars intermedia are described. In the first case the only symptom referable to the hypophysis was high blood pressure. On account of the age of this patient (77 years) the association of the adenoma with the hypertension is questionable. In the second case a number of the major characteristics of pituitary basophilism (adiposity, striae

atrophicae, hirsuties, high blood pressure, florid face) were present. In neither case were there any hyaline changes in the basophilic cells. There was considerable diffuse invasion of the neural lobe by these cells in both cases. The greater bulk of the data, however, does not indicate that there is any direct significant correlation between this invasion and hypertension. FROM AUTHORS' SUMMARY.

THE HISTOLOGY OF THE INFECTIOUS FIBROMA IN RABBITS. C. G. AHLSTROM, J. Path. & Bact. **46**:461, 1938.

The infectious fibroma of the rabbit shows both inflammatory and neoplastic features; the early stages are chiefly granuloma-like, whereas neoplastic features dominate the later stages. The cells originate not only from fibroblasts but also from perivascular histiocytes and endothelial cells, groups of young capillaries with hyperplastic endothelium serving as centers for the proliferation of the young tumor. The cells show characteristic basophilic cytoplasmic inclusions, increasing in amount and in size with the age of the tumor. The epithelium overlying the intracutaneous fibroma shows inclusions in the form of eosinophilic granules and sometimes also hyperplasia. Regression of the fibroma occurs through a combination of necrosis and resorption, of which the former seems to be primary. FROM AUTHOR'S SUMMARY.

PROPHYLAXIS OF OCCUPATIONAL CANCER. O. TEUTSCHLAENDER, Med. Welt **11**: 1267 and 1341, 1937.

Teutschlaender is the author of the so-called allobiotic theory of cancer, according to which the carcinogenic agent after a sufficiently long exposure produces in the body a condition of cancer readiness. That condition, or allobiosis, is characterized by the tendency of the body to react to irritating agents with neoplasia instead of with the usual reaction. A table presents all known instances of occupational cancer. Teutschlaender attempts to apply the three factors—(1) the constitution (allobiosis), (2) the carcinogenic agent and (3) the term of exposure—to the prophylaxis of occupational cancer. The applicability is illustrated in several industries, with particular consideration of the laws in Germany.

I. DAVIDSOHN.

TUMORS OF THE THYMUS AND THEIR ASSOCIATION WITH MYASTHENIA. R. A. OBIDITSCH, Virchows Arch. f. path. Anat. **300**:317, 1937.

Nine cases of tumor of the thymus are described. In 4 of the cases the growth was benign and was termed lymphoepithelial; the predominating cell was the small lymphocyte. In 5 cases the tumor was malignant and of the squamous epithelial type; this type the author terms a malignant medulla cell tumor. Only the benign tumors were associated with myasthenia. The benign tumors, which retained the function of the thymus, secreted an excess of a thymic substance causing myasthenia. Whether it acted directly on the muscle or on the nervous system or on the metabolism has not been determined. O. T. SCHULTZ.

VIRULENCE OF THE CELLS OF TRANSPLANTABLE TUMORS. A. SYMEONIDIS, Virchows Arch. f. path. Anat. **300**:429, 1937.

Inoculability in the case of a tumor is an expression of intrarelationship between the properties of the host and those of the tumor cell. Ehrlich, influenced by bacteriologic terminology, stated that the virulence of a transplantable tumor is composed of two factors: (1) its ability to overcome the host's protective mechanisms and establish itself; (2) its energy of growth after it has "taken." Inoculability can be measured by the percentage of "takes" in a series of animals of uniform con-

stitution. Unless "takes" in 100 per cent of cases can be assured, the variation in "takes" is too great to be of much value as a measure of virulence. In the work of Symeonidis with the Ehrlich transplantable mouse carcinoma and with the Ehrlich tumor originally termed sarcoma but which Symeonidis considers to be carcinoma of an anaplastic type, "takes" were obtained in 100 per cent of each series of inoculated animals. Inoculability is a fixed property. In the course of the work the so-called sarcoma became much more highly virulent as measured by rapidity of growth, appearance of metastases and duration of life. During this period "takes" were obtained with a dose of as few as 3,000 suspended cells. With 10,000 cells, "takes" were obtained uniformly. Such an increase in virulence must be due to increased resistance on the part of the tumor cells to the protective mechanisms of the host. Such an increase in virulence occurring in the course of a series of animal passages is due to segregation out of the tumor of cells more resistant or less susceptible to the antagonistic protective mechanisms of the host. In determining the degree of virulence of transplantable tumors it is necessary to determine the "absolute minimum" of living tumor cells that will result in a "take," and the "optimal minimum" of the smallest number of cells that will result in "takes" in 100 per cent of cases. Malignancy is not identical with virulence, as the author uses the latter term, and malignancy is not a measure of the virulence of transplantable tumors. Successful inoculation after implantation of organs that contain no visible tumor tissue is due to microscopic metastases or to cells of high virulence that have been carried into the organ.

O. T. SCHULTZ.

Medicolegal Pathology

DIAGNOSIS AND MEDICOLEGAL IMPLICATIONS OF ALCOHOLIC INTOXICATION. S. SELESONICK, J. A. M. A. **110**:775, 1938.

It is important to have definite criteria as a basis for the diagnosis of alcoholic intoxication in accidents involving persons who have imbibed alcoholic beverages. The chemical determination of alcohol in a body fluid offers a scientific means of establishing whether or not a person has imbibed alcohol and of estimating the degree of alcoholic intoxication. Blood as a medium for analysis is preferable to spinal fluid, urine, saliva or expired air for the following reasons: It contains a negligibly small amount of nonalcoholic oxidizable material. Its alcoholic content represents the degree of alcohol saturation at the moment the blood sample is obtained. It is always available, and its extraction does not necessitate the active participation of the subject.

There are sufficient scientific data to prove that subclinical intoxication—or alcoholic intoxication in the biologic sense without any gross manifestations of drunkenness—can produce sufficient interference with psychomotor activity and neuromuscular coordination to render the affected person a public menace. The technic of determining the alcohol in the blood detects degrees of alcoholic intoxication which ordinarily escape detection by competent physicians. Criteria, therefore, must be established which include body fluid alcohol determinations as part of the diagnostic armamentarium.

FROM AUTHOR'S SUMMARY.

ASPIRATION OF AMNIOTIC LIQUOR. J. CAMERER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **29**:333, 1938.

Reports of the presence of amniotic liquor in the lungs and bronchi of newborn children are exceedingly variable. After the publications of Haberda, the microscopic demonstration of even sparse elements from the amniotic fluid in the lung was accepted as the cause of natural death. Camerer has examined the lungs of 212 infants, in 45 of whom autopsy revealed evidence of aspiration of amniotic fluid. In 28 others the evidence was questionable, and in the remaining 139 no such evidence was found. Of these children, 93 were stillborn, 48 died during

the first day, 47 lived a day, and 24 died during the first week. In the majority of the cases, tissues from one lung only were prepared, since the author was unable to find any difference in the content of the various lobes. There was, however, a great variation in the localization of the material within a lobe. At times, the aspirated masses were located in the large and small bronchi. At other times, these structures were empty, and the alveoli were distended with foreign material. Of the 212 bodies examined, only 3 did not contain any demonstrable amniotic component, and in these 3 there was lymphocytic and leukocytic infiltration partly in the alveoli and partly in the interstitial tissue. In the 67 lungs in which only sparse epithelial cells were found and no fat, the cells were scattered in the alveolar spaces but were never found in the bronchi. These findings seemed unassociated with pulmonary function, as they were noted in lungs which had breathed, in those which had not breathed and in those which were macerated. It also made no difference if the child was immature or postmature, as their lengths varied from 32 to 56 cm. Because of the great constancy of these findings, the conclusion seemed justified that the process was physiologic.

In the lungs of 79 children, fat and epithelium were demonstrated in small or moderate amounts. It is remarkable that this aspiration has no after effects. In none of the author's cases was there an inflammatory reaction. It is evident, therefore, that these substances do not call forth a foreign body reaction or pneumonia. Large fat-laden wandering cells occurred in the alveoli involved. The rest of the fat was found in the endothelium of the alveolar capillaries in the form of fine droplets, especially in the older children. It appears, therefore, that the fat is taken up by the reticuloendothelial system of the lung and is there either changed or disposed of. The epithelial cells are preserved longer than the fat and are still stainable in macerated fetuses and in the lungs of children who have lived for eight days.

GEORGE J. RUKSTINAT.

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY

MAURICE N. RICHTER, *Vice President, Presiding*

Regular Meeting, Feb. 23, 1939

ROBERT A. MOORE, *Secretary*

GLOMUS TUMOR OF THE ARM. ANDREA SACCONE and JOSEPH MENDELOFF.

To date 106 cases of glomus tumor have been recorded in the literature. A case of glomus tumor in a 42 year old white man is described. The tumor occurred at the site of a "blue birthmark" after injury to that site. The "birthmark" was of ten years' duration, and the glomus tumor was of eight months' duration. The tumor cells appeared to be of myoblastic origin. The suggestion is made that there may have been a connection of these cells to the periglomal nerves by means of nonmyelinated fibers. In view of the fact that the exact nature of the previous "blue birthmark" is unknown, it is difficult to postulate a relationship of the glomus tumor to it, although some relationship is strongly suggested.

DISCUSSION

AMOUR F. LIBER: This case is of interest not only because of the situation, outside of the tips of the digits, which is somewhat unusual, but because of an unusual clinical feature, the fact that the pain was referred to the region of the sixth rib, which belongs to a dermatome entirely different from that of any part of the upper extremity. In most cases of glomus tumor the pain is referred very often to the same nerve or at least to the same dermatome. This opens a number of curious vistas, that the pain may be referred to a region of the skin innervated not by the same spinal metamere but possibly by some common sympathetic innervation. I have had occasion to observe a case of glomus tumor in the wrist which in some ways is analogous to this one (lantern slide shown). I think the lantern slide is sufficient to demonstrate that one is dealing with a glomus tumor. The marker represents a length of 100 microns. The pain in this case had lasted ten years but was slight. Here again is a history of trauma, apparently slight, but sufficient to cause an ecchymosis in the painful region, and following this the pain was aggravated, and a visible growth appeared.

There is one other point. Very often small painful bluish tumors which have the same subjective features as glomus tumors are found in the extremities and on the body, particularly on the thighs and legs, and are frequently diagnosed clinically as glomus tumors. Often they are painful subcutaneous leiomyomas, to which Dr. Stout has called attention in his study, and of course they are easy to diagnose histologically. Clinically they are almost invariably called glomus tumors.

ANDREA SACCONE: I should like to add that the glomus tumor is a type of angioma, a complex type, in which the neuromyoblastic elements play an important role in the clinical manifestations and in the histologic appearances.

EFFECTS OF INTRAVENOUSLY INJECTED SUCROSE OF THE KIDNEYS. PAUL KLEMPERER.

In routine examinations of autopsy material of the past two years it was noted that a most striking vacuolation of the cytoplasm of the epithelium of the pri-

mary convoluted tubules of the kidneys was being encountered much more frequently than in preceding years. In an investigation to determine whether any therapeutic procedure might have been responsible for this conspicuous alteration it was found that every one of the patients had received one or more intravenous injections of 50 per cent sucrose solution. These observations are not original, because identical findings in animal experiments have been reported by Lamy, Mayer and Rathery and by Helmholtz, who noted also similar changes in a case in man. The object of the demonstration is to call attention to these striking changes, which have been found frequently and are puzzling.

DISCUSSION

THEODORE BAUER: When I saw the pictures presented by Dr. Klemperer, I remembered the little spots frequently found in all kinds of kidneys; they have been described by Stoerk. They resemble the cortical cells of the adrenal by their big size, their vacuolated protoplasm and their plantlike appearance, so that Stoerk tried to explain, in opposition to Grawitz, the hypernephroma not as a derivative of adrenal rests but, corresponding to these large kidney cells, as nephrogenic tumor. Sometimes one can see only a few cells showing this peculiar degeneration; sometimes, quite a group of tubules with the same changes. The vacuolation may be caused by deposition of lipoid or of glycogen. I wish only to draw attention to the point that these peculiar cells, resembling the cells of the cortex of the adrenal gland, caused in the cases just demonstrated by sucrose, follow Stoerk's theory as to the base from which the Grawitz tumor (hypernephroma) cells originate.

IRVING GRAEF: Were these cells observed in normal kidneys?

PAUL KLEMPERER: Years before in routine material I probably observed similar changes, because one sees vacuolation in some severe infections—for instance, in chronic dysentery. Vacuolar degeneration is not too uncommon. It was the frequency with which one encountered this change which was so striking that one had to look for some cause. I think the cause was the sucrose injected, not only because injection of sucrose was found in our cases but also because in experimental investigation an exactly identical change had been found. I want to emphasize the fact that these vacuoles are not lipids nor are they due to deposition of glycogen.

IRVING GRAEF: The significance of my question had to do with the fact that Dr. Klemperer showed pathologic kidneys. Dr. Klemperer may recall a case I have been studying, which was referred to me through Dr. Jacob Werne. It concerned a child who had renal insufficiency for no apparent reason and who at autopsy had markedly swollen kidneys, with hemorrhage into the pelvis and around the pelvis, and, in addition, microscopic thrombosis of the renal vein. The etiologic explanation of this thrombosis was never established. In addition to these abnormalities, the tubules of the kidney everywhere exhibited the same change which Dr. Klemperer showed; the nuclei were normal. I thought there might have been some disturbance in the metabolism or in the excretion of urine which promoted storage of a substance that would ordinarily diffuse out, and I too found that there was no lipid in the epithelium. The point which perplexed me was: Could there be storage of a polysaccharide like sucrose in a normal epithelial cell to the extent which was demonstrated? Must renal insufficiency be present to promote such storage, or some local change in function which would promote such storage? I have had the opportunity of examining the kidneys from animals which had received large amounts of inulin, which ordinarily diffuses through the glomerular membranes, and in these animals I have never seen anything to compare with this change. I wonder whether Dr. Klemperer feels that a local disturbance is necessary to promote such a tendency toward storage of sucrose.

AMOUR F. LIBER: Dr. Klemperer's paper will undoubtedly be extremely enlightening. Just a few days ago I examined slides of a kidney from a child

who had died after prolonged diarrhea, and I recall now that the kidney, which presented no other gross or microscopic lesion, showed a diffuse vacuolar condition of the epithelium of the convoluted tubules, which, it seems to me, must have corresponded to the picture which Dr. Klemperer showed. The child had not received sucrose, which has not been used at the Lincoln Hospital currently, but had received large amounts of dextrose intravenously and perhaps saline solution, but certainly dextrose. I wonder whether this might not be a phenomenon of water accumulation.

PAUL KLEMPERER: In regard to the question whether the vacuoles contain sucrose, I cannot say that they do not, but what is known of the excretion of sucrose speaks against it. Sucrose is excreted by the normal kidney within seventy-two hours quantitatively. Dextrose in very high, 50 per cent, concentrations, also produces vacuolation, though not as striking vacuolation as in these cases. Less concentrated solutions apparently do not produce change.

In regard to Dr. Graef's question: Some of the patients were perfectly normal as far as renal function was concerned. The same feature was shown in a case of subacute bacterial endocarditis with cerebral embolism in which sucrose had been injected. There was also a case of heart failure due to myocardial fibrosis in which there was no renal insufficiency, and the same picture was found. Moreover, the experiments made by Helmholtz showed an identical picture in normal rabbits. I cannot recall exactly the case to which Dr. Graef called my attention, but I may mention 2 cases of renal insufficiency in which there was vacuolation of the severest type with no suspicion of an injection of sucrose, because sucrose therapy was not known at that time. The histologic picture was different. The tubular cells were destroyed to a much greater extent than in the instance reported here, in which the epithelial cell seems intact and shows only striking vacuolation.

PATHOLOGIC CHANGES FOLLOWING THERAPEUTIC HYPERTHERMIA. LOUIS LICHTENSTEIN (by invitation).

A description is given of changes observed at necropsy in a case of uncontrollable hyperpyrexia (109 F.) ensuing on hyperthermic treatment for arthritis of the finger joints. The hyperpyrexia (which developed in the course of the third of a series of treatments) was associated with coma, hemiplegia and respiratory failure, and the subject died about thirty-five hours after the fever was initiated. In this case the pathologic changes were as follows: (1) multiple punctate hemorrhages and necrobiosis in the gray matter of the cerebral cortex; (2) hemorrhage in the left internal capsule; (3) thrombosis of venules and capillaries in the cerebral cortex and internal capsule; (4) cerebral congestion and edema; (5) infarction of kidneys and spleen; (6) marked hepatic degeneration and edema; (7) pulmonary congestion, hemorrhage and edema.

The observations in this case are correlated with, and discussed in relation to, the observations at autopsy in 9 other instances of fatal fever therapy reported within the past few years. Certain of the features presented—notably the vascular lesions—have not hitherto been described in connection with fatalities following fever therapy. Specifically, there seems to be no previous description of the thrombosis of venules and capillaries in the hemorrhagic portions of the brain or of the infarcts in the kidneys and spleen, which apparently were the result of focal necroses of small arterial branches in these organs.

The principal complications and sequelae of hyperthermia, and especially its effects on the brain, blood vessels and liver, are indicated.

DISCUSSION

MAURICE N. RICHTER: I should like to ask whether the vacuoles in the liver contained eosinophilic bodies. From the photograph they resembled the

structures described years ago by Mallory and more recently by Pappenheimer and Hawthorne (*Am. J. Path.* **12**:625, 1936), and they can be found in a rather high percentage of livers in routine examination.

LOUIS LICHTENSTEIN: No eosinophilic bodies were noted microscopically.

ACUTE POSTOPERATIVE ENTEROCOLITIS: A STUDY ON THE PATHOLOGIC NATURE OF SHOCK. ABRAHAM PENNER (by invitation) and ALICE BERNHEIM (by invitation).

This paper will be published in full in a later issue of the ARCHIVES.

CHICAGO PATHOLOGICAL SOCIETY

KATHARINE M. HOWELL, *President*

Regular Monthly Meeting, March 13, 1939

EDWIN F. HIRSCH, *Secretary*

FIBROSARCOMA OF SOFT TISSUE WITH REGIONAL CONCENTRIC ABSORPTION OF BONE NOT DUE TO THE TUMOR. ORMAND C. JULIAN.

The clinical and pathologic details of a slowly growing fibrosarcoma of the soft parts of the forearm are reviewed. The history of the disease from the appearance of the tumor until the amputation of the arm extended sixteen years. The patient had no metastases nine months after the amputation. An unusual feature was the concentric absorption of bone in the radius and ulna which resulted in pathologic fractures. These changes were present at least four years before gross or microscopic evidence of extension of the tumor into the bone was found. The histologic appearance of the tumor tissues was not characteristic of sarcoma. However, regional metastases to the skin of the forearm indicated malignancy.

DISCUSSION

D. H. PHEMISTER: The behavior of the soft tissues was peculiar, and the diagnosis of sarcoma was not simple, because of the long duration of the disease. The disturbance in the bone was an external concentric absorption of the radius and then the ulna. When the radius was first exposed, there was no evidence of tumor in the periosteum. Later the tumor tissues were in the bone. It seems to be a tumorous disease of long duration in the soft parts with spread into the bone.

O. SAPHIR: I have seen several tumors like this, arising in or near the periosteum. Is there evidence that this is a slowly growing neurofibroma? The neurofibroma is radioresistant. Later the growth became sarcoma with invasion of the bone.

O. C. JULIAN: No special stains for the tissues of neurofibroma were made, but palisades of cells were not a special feature. There were no metastases.

KODACHROME FILMS FOR TEACHING PATHOLOGY. S. A. LEVINSON, W. O. BROWN and J. R. THOMPSON.

The advantages of Kodachrome films of fixed and unfixed pathologic tissues for teaching purposes were illustrated and discussed.

HISTOLOGY OF THE PITUITARY OF THE WHITE RAT AFTER INJECTION OF AN ESTROGEN. ARTHUR WEIL and BERNHARD ZONDEK.

Injections of dimenformon (estradiol benzoate) into white rats twice each week over long periods produced the following changes in the pituitary: With

120,000 mouse units, administered during nine to twelve weeks, the anterior lobe was enlarged to about one and a half times its normal size. There was mild swelling of the cells of the three different types. The proportion of eosinophilic cells was 30 to 35 per cent as compared with 38 to 32 per cent in controls given injections of olive oil. With 600,000 mouse units, injected during seventeen to thirty weeks, the anterior lobe was enlarged to about twice its normal size. There was a mild increase in its vascularity. In all of the three types of cells, which were moderately swollen, the Golgi apparatus was markedly enlarged and contained a coarse granular débris. The proportion of eosinophilic cells was diminished to 21 to 28 per cent. With 780,000 mouse units, injected during thirty-two to sixty-three weeks, the anterior lobe was three to four times its normal size and attained a maximal weight of 100 mg. The interlobular cleft was markedly widened. There was a marked increase in vascularity, which in some rats led to intralobular hemorrhages and death. The cells of all the three types were swollen, the Golgi apparatus was maximally dilated, and most of the chromophils were without granules. The pars intermedia and the pars nervosa were not directly affected by the injected estrogenic drug. In the last two groups they were compressed by the enlarged anterior lobe. In the cases in which the changes were advanced the pars nervosa had a loss of cells and an increase in glia fibers. Compression of the hypothalamus led to atrophy of the median eminence and of the supraoptic nuclei. Compression of the anterior cerebral arteries was followed by thrombus formation and softening in the frontal lobes.

PRIMARY FIBROMYXOMA OF THE HEART. HOWARD G. BENJAMIN.

All tumors of the heart are rare, but primary tumors are much less frequent than secondary tumors, the ratio being about 1 : 16. Secondary tumors, observed in 0.5 per cent of 40,000 necropsies, affect the right side of the heart more frequently than the left. Primary tumors, observed in 0.03 per cent of 40,000 necropsies are more commonly benign than malignant, the ratio being about 3 : 1. They involve the left side of the heart more often than the right. The most common primary tumor is the myxoma (including fibromyxoma, elastomyxoma and other types), which comprises about 45 per cent of all primary cardiac tumors. It occurs at any age and about equally with respect to the sexes. The left auricle, especially the interauricular septum in the region of the fossa ovalis, is the usual site. The tumor frequently forms a pedunculated intra-auricular mass, which may produce a ball valve obstruction of the mitral orifice. The symptoms and signs of any cardiac tumor, primary or secondary, depend on the size and the location of the growth with special reference to the conduction mechanism of the myocaridum and to the orifices of the valves and of the great vessels entering the heart.

A white youth aged 17 complained of increasingly severe dyspnea and of generalized weakness. Hemoptysis, fainting spells and edema of the ankles occurred. His illness followed an infection of the upper respiratory tract and lasted three weeks. He had dyspnea, cyanosis, an apical presystolic thrill and murmur, an apical systolic murmur transmitted to the axilla, an accentuated pulmonic second sound, a pulse rate of 132 with a deficit of 12, and signs of heart failure. Death occurred suddenly eleven hours after he had entered the hospital. Autopsy demonstrated a pedunculated cellular fibromyxoma of the left auricle, producing ball valve obstruction of the mitral orifice and the changes of cardiac decompensation, such as hydrothorax, hydropericardium, pulmonary edema and passive hyperemia of the lungs, liver and spleen. Microscopic examination revealed no evidence of rheumatic or other disease of the heart.

DISCUSSION

H. G. BENJAMIN: Metastatic growths of the heart are usually in the right ventricular tissues, probably owing to the vascular distribution.

Book Reviews

Manual of Veterinary Bacteriology. Raymond A. Kelser, D.V.M., A.M., Ph.D. Third edition, thoroughly revised. Cloth. Pp. 640, with 93 illustrations. Price $6. Baltimore: Williams & Wilkins Company, 1938.

That Kelser's Manual has proved a useful addition to the limited list of satisfactory veterinary textbooks is attested by the fact that the present volume is the third edition in ten years.

The contents are presented in well ordered sequence. After a concise introductory chapter on the history of bacteriology, the author takes up the morphology, physiology and classification of bacteria. The classification is that proposed by the Society of American Bacteriologists, as incorporated in the 1934 revision of Bergey's Manual.

Chapters 3 to 7, inclusive, deal with the microscope, sterilization, preparation of culture mediums, methods of artificial culture and the staining and microscopic study of bacteria. Next, the basic principles of immunization and hypersensitiveness are described. The tuberculin reaction, however, is barely mentioned. The importance of the tuberculin test in veterinary medicine leads one to expect at least a brief discussion of the theories concerning this phenomenon. Nor are references to literature in which the student might find this information given.

The chapter on bacterial variation is competently written; the impressive list of references indicates that the recent literature has been surveyed. The dissociation of Mycobacterium tuberculosis should have been included in the consideration.

Twenty chapters are then devoted to the pathogenic bacteria of etiologic significance in diseases of the lower animals. The pathogens responsible for important infectious diseases of human beings are not described, since the author considered such bacteria outside the scope of the text. Generally speaking, the respective species of bacteria are adequately considered for classroom purposes, but there are some shortcomings. The most important of these is the failure to bring portions of the text into conformity with the more recent literature. For instance, exceptions might be taken to the statement that a number of strains of Erysipelothrix rhusiopathiae "will produce small amounts of acid from glucose and lactose" when the data available indicate that all strains of this organism produce acids from these carbohydrates. Although the salient facts concerning Brucella abortus are adequately considered, the pathogenicity of Brucella suis (except for guinea pigs) should have been stressed more. The fact that this organism occasionally produces lesions in swine and the possibility that employees of slaughterhouses may contract brucellosis from infected carcasses of swine are of importance to the public health and should have been discussed at least briefly. In the consideration of Actinomyces necrophorus, exception should be taken to the statement that this organism is the cause of so-called lip and leg ulceration of sheep. Observations by competent investigators (1934 and before) indicate that the lesions of the mouth, at least, are due to a filtrable virus, with A. necrophorus playing the role of a secondary invader. In fact, the evidence available raises doubts whether or not A. necrophorus is ever the primary factor in many of the lesions in which it is found.

In the section on Myco. tuberculosis it is stated that tuberculosis of dogs and cats is usually due to the human type of organism; as a matter of fact, dogs are probably equally susceptible to the bovine organism, and the type of infection acquired depends on the circumstances of their exposure. Whether cats are susceptible to the human type of infection is problematic. In the cases of tuberculosis of the cat reported, the disease has been due without exception to the bovine organism.

Among the methods suggested for the isolation of Myco. tuberculosis the newer procedures are not mentioned, and in considering the cultural requirements of the bovine form of the organism it is inferred that a glycerinated medium is satisfactory. Experience in isolating this organism from the tissues of naturally infected cattle indicates definitely that most bovine strains of Myco. tuberculosis are nonglycerophilic. The statement that in tuberculous chickens pulmonary involvement is quite infrequent is likewise at variance with many observations. In view of the marked reduction of tuberculosis in cattle during the past decade, one might well disagree with the author in his opinion that the bovine type of Myco. tuberculosis is the common cause of tuberculosis in swine. The reports of several investigators indicate that the avian type of the organism is responsible for a large proportion of the tuberculous disease in swine. A discussion of heterologous sensitization to tuberculin would also have been of value.

Four chapters are devoted to the pathogenic fungi, and there are 53 well written pages on the protozoa, including the technical methods best suited to the study of these organisms. It is regrettable, however, that Trichomonas foetus was not included.

A chapter deals with the various filtrable viruses. This chapter contains a large amount of information on an increasingly important group of animal diseases. The subject is ably presented, and the conclusions are conservative.

The last 4 chapters deal with practical serologic tests and other methods for clinical examination of the blood. A consideration of the blood of the domestic chicken is not included. There are also descriptions of methods for the preparation of biologic products used in veterinary medicine and of the standard methods for bacteriologic examination of milk and water.

The index is adequate. A check of 30 bibliographic references taken at random revealed a few errors of minor importance. The size of the edition has been increased by 88 pages.

While this book has certain shortcomings, the desirable qualities outweigh any deficiencies or errors. The new edition merits a warm reception by teachers and students of veterinary medicine.

Les meningo-neurobrucelloses. Henri Roger and Yres Poursines. Paper. Pp. 248. Price 65 francs. Paris: Masson & Cie, 1938.

The senior author of the book is an outstanding authority on brucelliasis as it affects the nervous system. Since 1923 he and his collaborators have published twenty-seven papers on this subject. The increasing number of reports dealing with effects of brucelliasis on the nervous system published in recent years indicates that such forms of the disease either passed unobserved or were given little consideration in the past.

The first chaper of the book is devoted to the general conception of the problem of brucelliasis in animals and in man. One will not find here a very complete and up-to-date review of this phase of the subject. However, the discussion pertaining to the clinical manifestations of the disease in man is worthy of study. In the succeeding ten chapters the authors have brought together the published observations scattered through the literature and combined them with their own extensive observations, with the result that there is here afforded a comprehensive analysis of the effects of Brucella infection on the central and peripheral nervous system. The effects of the disease on each of the principal anatomic parts of the nervous system are discussed in a separate chapter. Due consideration is given to the clinical diagnosis, the prognosis and the therapeusis of nervous forms of the disease. The authors note that the neurologic manifestations seldom if ever appear early in the disease; that the clinical symptoms may present a changing pattern. A large number of the severe forms terminate fatally; others may be observed as mental disturbances of indefinite duration.

The information contained in the book should be a valuable guide to those interested in the neurologic aspects of the disease. It is obvious that one should

not neglect to consider brucelliasis when the causes of a disease of the nervous system are sought for in many patients. There is appended a bibliography containing 238 references, most of which pertain to published observations on neurobrucelliasis.

Pathologische Histologie: Ein Unterrichtskurs für Studierende und Ärzte. Dr. Max Borst, Professor der allgemeinen Pathologie und der pathologischen Anatomie an der Universität München. Third edition. Paper. Pp. 522 with 361 illustrations. Price 75 marks. Berlin: Julius Springer, 1938.

The first edition of this book was published in 1922; the second, in 1926. It grew from 371 pages to the present size. The number of illustrations rose from 240 in the first edition to 275 in the second and to 374 in the present. On the title page mention is made of 361 illustrations but in the text this number of illustrations is increased by 13 insertions. All but 19 illustrations are colored. The introduction is a profound, fascinating philosophic analysis of histopathology and of the part played by the histologist in the practice of medicine. The subject is treated according to anatomic principles, in 10 chapters but for the neoplasms, which are presented collectively in the final (eleventh) chapter, containing 137 pages. The first 2 chapters deal with the organs of circulation and with the blood and blood-forming organs; then follow chapters on the organs of respiration and digestion, on the urinary, genital and nervous system, on organs of locomotion, on the skin and on the glands of internal secretion. Each chapter opens with a concise but clear presentation of the normal histology. While histopathology is stressed, the general pathology and pathogenesis of each subject are considered as exhaustively as the size and purpose of the volume permit. The microscopic changes are correlated with the gross pathologic observations and frequently also with clinical manifestations. The pictures were drawn by W. Freytag from original preparations; they are well chosen and excellently executed. A valuable feature is the thorough textual analysis of minute details of the pictures. Recent developments are discussed. There are no references. The paper and print are good. A well organized index fills 15 pages. The book can be recommended warmly to all students of histopathology.

The Chemistry of Antigens and Antibodies. By J. R. Marrack, D.S.O., M.C., M.D. Paper. Pp. 194, with illustrations. Price 3 shillings. Medical Research Council, Special Report Series, no. 230. London: His Majesty's Stationery Office, 1938.

This monograph is a new edition of special report 194, revised and enlarged. The aim in this, as in the first edition, is to apply physicochemical principles and methods to the study of the nature of immune reactions. The general plan of the book remains the same. It contains, as before, five chapters, each of which has been extensively revised. In the first chapter, on physicochemical considerations, the main change is in the discussion of recent theories of protein structure. Chapters 2 and 3, on the nature of antibodies and the specificity of antigens, respectively, have been considerably enlarged and brought up to date. The most extensive additions have been made to chapters 4 and 5, on the nature of antigen-antibody reactions. This volume, like its predecessor, represents the best attempt this reviewer has seen to apply chemical and physicochemical principles to the solution of immunologic problems. It should prove of great value in stimulating students of immunology to apply physicochemical methods to the study of the mechanism involved in antigen-antibody reactions. There are extensive references to the original literature for those interested in following the advances being made in this fascinating field.

Books Received

CLINICAL BIOCHEMISTRY. Abraham Cantarow, M.D., Associate Professor of Medicine, Jefferson Medical College; Biochemist, Jefferson Hospital. Max Trumper, Ph.D., Clinical Chemist and Toxicologist; formerly in charge of the Laboratories of Biochemistry of the Jefferson Medical College and Hospital. With a foreword by Hobart A. Reimann, M.D., Professor of Medicine, Jefferson Medical College. Second edition, revised. Cloth. Pp. 666. Price $6. Philadelphia: W. B. Saunders Company, 1939.

THE GENERAL TISSUE AND HUMORAL RESPONSE TO AN AVIRULENT TUBERCLE BACILLUS INCLUDING GROWTH CHARACTERISTICS OF THE ORGANISM. Sol Roy Rosenthal, M.D., Ph.D., Associate in Bacteriology and Public Health. Joint Contribution from the Tice Laboratories of the City of Chicago Municipal Tuberculosis Sanitarium and the College of Medicine of the University of Illinois. Illinois Medical and Dental Monographs, vol. 2, no. 2. Paper. Pp. 184, with 80 illustrations. Price $2.50. Urbana: University of Illinois Press, 1938.

CRYSTALLINE ENZYMES. THE CHEMISTRY OF PEPSIN, TRYPSIN, and BACTERIOPHAGE. John H. Northrop, Member of the Rockefeller Institute for Medical Research. Cloth. Pp. 176, with 48 illustrations. Price $3. New York: Columbia University Press, 1939.

ANGINA PECTORIS. NERVE PATHWAYS, PHYSIOLOGY, SYMPTOMATOLOGY, AND TREATMENT. H. R. Miller, M.D., Attending Physician, Sydenham Hospital; Associate Attending Physician, Montefiore Hospital, New York City. Cloth. Pp. 275, with 39 illustrations. Price $3.25. Baltimore: William Wood & Company, 1939.

FAILURE OF THE CIRCULATION. Tinsley Randolph Harrison, M.D., Associate Professor of Medicine, Vanderbilt University, School of Medicine, Nashville, Tenn. Second edition, revised. Cloth. Pp. 495, with 60 illustrations. Price $4.50. Baltimore: Williams & Wilkins Company, 1939.

BY-EFFECTS IN SALVARSAN THERAPY AND THEIR PREVENTION WITH SPECIAL REFERENCE TO LIVER FUNCTION. V. Genner. Paper. Pp. 360. Copenhagen: Levin & Munksgaard, 1936.

TWENTY-NINTH ANNUAL REPORT OF THE CHARLES V. CHAPIN HOSPITAL, PROVIDENCE, R. I., FOR THE YEAR ENDING SEPTEMBER 30, 1938. Paper. Pp. 82. Providence: The Oxford Press, 1938.